VOLUME 90 NUMBER 5

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

May, 1952

Effect of 17-Hydroxycorticosterone-21-Acetate on Growing Bones of the Rat — Richard H. Follis, Jr. 337

The Renal Excretion of Histamine and Histidine in Man and the Effect of Adrenocorticotropic Hormone (ACTH) and Cortisone Administration. — David Grob 341

Failure of Podophyllin in the Treatment of Cutaneous Carcinoma. — Maurice Sullivan, Laurence Zell and Hugh McCulloch, Jr. 368

The Relation of Dosage Schedule to the Therapeutic Efficacy of Terramycin. — Louis Lasagna and C. G. Zubrod 372

Proceedings of the February Meeting of the Johns Hopkins Medical Society 382

Book Reviews 387

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

Copyright, 1952, by The Johns Hopkins Press

BULLETIN OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, FREDERIK B. BANG

ASSOCIATE MANAGING EDITOR, CHARLES G. ZUBROD

EDITORIAL BOARD

E. Cowles Andrus **Kennneth C. Blanchard** **John Eager Howard**
Charles R. Austrian **Edward M. Hanrahan** **Arnold R. Rich**

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75.

New subscriptions and renewals are entered to begin with the first issue of the current year. Subscribers in the United States are hereby notified that missing numbers cannot be replaced unless notification of loss is received in this office within 1 month following publication of the missing number; foreign subscribers 6 months.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

Manuscripts. Manuscripts should be typewritten, one side of page only, and double-spaced. Following the title should appear the author's name, department, and the university and/or hospital he represents. A list of references (bibliography) in numerical sequence should appear at the end of article. Preferred style for references is as follows: Author's last name, initials. Title of book or article, with first word and proper names capitalized only. Name of journal (abbreviate), date of publication, volume number: page number. The Bulletin is not responsible for loss of a manuscript in mail. Author should retain a duplicate copy.

Illustrations and Tables. Legends are required for Figures and descriptive headings for Tables. Use Arabic numerals to number Figures (Fig. 1); use Roman numerals to number Plates and Tables (Plate I). It should be indicated exactly where Figures and Tables are to appear in text; Plates all appear at end of article. All Figures should be drafted in black India ink for 50% reduction to $4\frac{1}{2}$ inch width. Glossy photographs must be supplied for all Plates. Original illustrations will be returned to author with galley proofs.

Reprints. Twenty-five reprints, without covers, are furnished gratis for each article. Additional reprints should be ordered when galley proofs are returned.

THE JOHNS HOPKINS PRESS

BALTIMORE-18, MARYLAND, U. S. A.

Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

EFFECT OF 17-HYDROXYCORTICOSTERONE-21-ACETATE ON GROWING BONES OF THE RAT

RICHARD H. FOLLIS, JR.

Department of Pathology, The Johns Hopkins University School of Medicine

Received for publication February 11, 1952

The administration of suitable amounts of cortisone acetate to young rats leads to marked changes in the skeleton (1). Such alterations consist of a broad zone of increased density at the growing ends of the long bones and costochondral junctions due to an increased amount of calcified cartilagenous matrix encased in bone. This change has been interpreted as resulting from a disturbance in normal osteolytic activity, osteoblastic sequences going on as usual. It seemed, therefore, of interest to study the effects of another closely related steroid, 17-hydroxycorticosterone-21-acetate* (Compound F) on the growing bones of the rat.

EXPERIMENTAL

The hormone was administered intramuscularly daily to young rats (McCollum strain) weighing 45 to 50 grams at the beginning of the experiment. Since we had found that to produce skeletal lesions the most effective daily dose of cortisone was 50 mg. per kilo per day, this amount was chosen in the first series of animals. Subsequent groups were given 25, 10, and 5 mg. per kilo per day. Each series consisted of 6 rats which were killed at intervals up to 24 days. The bones were processed for histological examination by standard procedures.

RESULTS

When the hormone was administered in daily doses of 50 mg. per kilo, a dense zone of calcified matrix encased in bone appeared at the ends of the growing bones. This was identical to that which had been observed when cortisone was given in like amounts (1). The daily dosage was decreased to 25 mg. per kilo; the effects were entirely the same. At a level of 10 mg. per kilo per day the dense zone was again present though the amount of calcified matrix and bone was not so great. When this dose was halved to 5 mg. per kilo the change, which was minimal, was only seen in animals sacrificed at 21 and 24 days.

DISCUSSION

It would seem that 17-hydroxycorticosterone-21 acetate has the same effect on the growing bones of the rat as does cortisone. It is of interest, however, that a difference in activity appears to exist. When cortisone was administered in

*Furnished through the kindness of Merck & Co., Rahway, New Jersey.

daily doses of 2.5, 10, and 20 mg. per kilo per day, no effects could be recognized (1). It was not until amounts of 40 or 50 mg. were injected that an effect on the skeleton could be elicited. Over the above quantities cortisone had a retarding effect on growth. It would appear then that 17-hydroxycortisone-21 acetate is more active in producing this specific change than is cortisone, since smaller daily doses of the former can elicit the alteration. It should be pointed out that the rat seems to be unique among the species thus far studied in its reaction to cortisone. Mice, guinea pigs, and rabbits do not exhibit skeletal lesions when injected with similar amounts of cortisone (2).

SUMMARY

Skeletal lesions similar to those produced by cortisone may be observed when 17-hydroxycorticosterone-21 acetate is administered to growing rats. The latter is more effective quantity for quantity than the former.

REFERENCES

1. FOLLIS, R. H., JR.: Effect of cortisone on the growing bones of the rat, Proc. Soc. Exp. Biol. and Med., 1951, **76**: 722.
2. FOLLIS, R. H., JR.: Non-effect of cortisone on the growing bones of mice, guinea pigs and rabbits, Proc. Soc. Exp. Biol. and Med., 1951, **78**: 723.

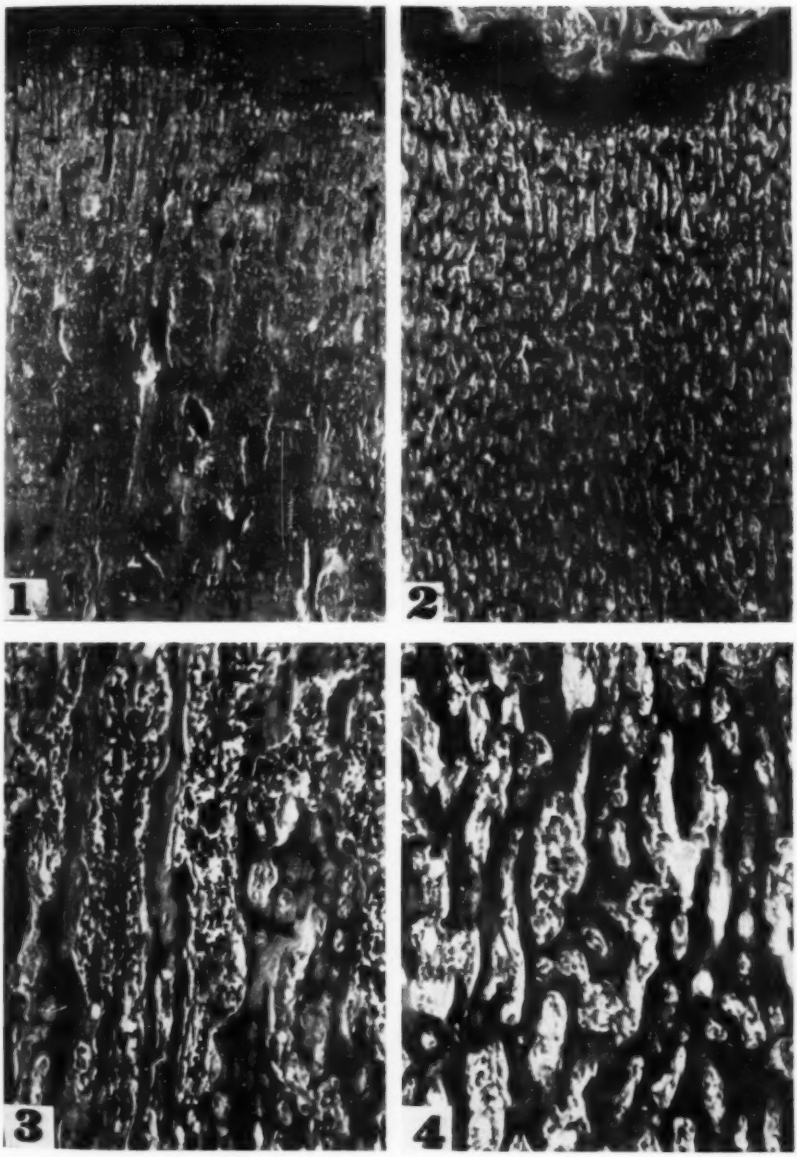
EXPLANATION OF PLATE I.

FIG. 1. Upper end of tibia of control rat sacrificed at 24 days of age.

FIG. 2. Upper end of tibia of rat receiving 50 mg. 17-Hydroxycorticosterone-21-acetate for 24 days. Note increased number of trabeculae in primary spongiosa. These trabeculae consist of calcified cartilagenous matrix encased in bone.

FIG. 3. High power of primary spongiosa of section shown in Figure 1.

FIG. 4. High power of primary spongiosa of section shown in Figure 2. (Same magnification as Figure 3.)


1
2
3
4

THE RENAL EXCRETION OF HISTAMINE AND HISTIDINE IN MAN, AND THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) AND CORTISONE ADMINISTRATION[1]

DAVID GROB

with the assistance of BARBARA ZIEGLER and GRACE SALTZER

Department of Medicine, The Johns Hopkins University and Hospital

Received for publication February 27, 1952

Histamine is normally present in very small amounts in the blood, chiefly in conjugated form within the white blood cells (1). It has been reported to be increased in a minority of patients with allergic disease (2). The conjugated form of histamine is a normal constituent of urine (3), and is said to be excreted in increased amount in some patients with bronchial asthma or other forms of hypersensitivity (4–6), and following a protein-rich diet (3). The mechanism of excretion is not known. Following the administration of adrenocorticotropic hormone (ACTH) to patients with bronchial asthma, the urinary excretion of histamine, as determined by biologic methods, has been reported to decrease in some patients (4, 5) and to increase in others (6).

Histidine is a normal constituent of plasma. It has been reported to be decreased in patients with rheumatoid arthritis (7), and to increase in these patients during treatment with ACTH (8). Histidine is present in the urine of most normal subjects (9, 10). The mechanism of its excretion in man has not been studied, but observations in experimental animals have indicated that it is excreted by a mechanism involving renal tubular reabsorption of almost all the compound calculated to be in the glomerular filtrate (11). There is no evidence that histidine can be secreted by the renal tubules.

Increased histidinuria has been observed during most normal pregnancies (9, 10, 12), during remission of rheumatoid arthritis, whether occurring spontaneously or following ACTH or cortisone (8, 13), following the administration of ACTH to patients with bronchial asthma (4–6), and possibly following a protein-rich diet (10). The cause of the increased histidine excretion induced by ACTH and cortisone is not known. It has been attributed to increased plasma concentration of histidine (8), and the suggestion has been made that it may be the result of remission of the disease under treatment (8, 13). Some evidence has been presented that it is not due to a lowered renal threshold for histidine (14).

Although intestinal bacteria are capable of converting histidine to histamine

[1] The work described in this paper was supported by a contract between the Medical Division, Chemical Corps, U. S. Army and the Johns Hopkins University.

by decarboxylation (15), the interrelation of these substances in the body is poorly understood. Their role, if any, in allergic diseases and in diseases of mesenchymal tissue in which hypersensitivity may play a part is not known. However, it has been reported that the administration of adrenal cortical hormone to adrenalectomized rats restores resistance to histamine poisoning (16) and ability of tissues to metabolize histamine (17). These observations, and the reported effect of ACTH administration on urinary excretion of histamine and histidine in man, have led to the suggestion that the action of ACTH and cortisone in allergic and "hypersensitive" states may be mediated by alteration of the "histidine-histamine balance" (4, 5, 18).

The study to be reported was undertaken in an effort to determine if allergic and "hypersensitive" diseases, and their response to ACTH and cortisone administration, are associated with any alteration in the disposition of histamine or histidine. This was investigated by comparing the blood level and urinary excretion of histamine and histidine of patients with these illnesses with that of normal subjects and of patients with other chronic illness, and by observing the effect of ACTH and cortisone. The occurrence of increased urinary excretion of histidine during the administration of these hormones was confirmed. The factors responsible for this phenomenon were investigated by study of the mechanism of renal excretion of endogenous and injected histidine, the renal and extrarenal components in the disappearance of injected histidine, and the influence of ACTH and cortisone on these processes.

METHODS

The concentration of histamine in the blood cells and urine was determined colorimetrically, after extraction with methyl isobutyl ketone of the colored azo compound formed with 4-nitrodiazobenzene, according to the method of Rosenthal and Tabor (19). This method determines both free and conjugated (acetylated) histamine in concentrations above 0.3 μg./ml., but is not sufficiently sensitive to determine concentrations that have been reported by biologic methods to be present in normal whole blood (0.02 to 0.07 μg./ml., (2)).

The concentration of histidine was determined colorimetrically, in the urine by the bromination method of Knoop (20), as modified by Langley (9), and in the serum by the diazotization method of Pauli (21), modified to employ p-chloroaniline (22). There is evidence that the resulting colors are due primarily to histidine, although they may be influenced by other compounds, and may vary with pH and temperature (9, 10, 12, 20). In order to minimize these variations, serum and urine obtained after the administration of ACTH or cortisone, and after the intravenous infusion of histidine, were analyzed simultaneously with specimens which had been obtained prior to these procedures and which had been stored in the frozen state. It was observed that histamine, acetyl histamine, and histidine added simultaneously to urine or blood were quantitatively recovered by these methods. Histamine, acetyl histamine, and inulin did not interfere with the determination of histidine, nor did histidine interfere with the determination of the former compounds. The presence of heparin affected the color of the azo dye formed with histidine, so that serum rather than heparinized plasma was employed for the determination of this com-

pound. Concentrations of histamine and histidine have been expressed in terms of the free base.

The rates of glomerular filtration, tubular reabsorption, and urinary excretion of histidine, and the rate of disappearance of injected histidine from the plasma, were studied in one male and two female patients who had no evidence of renal disease. Two of the patients had myasthenia gravis and one had cold allergy. Renal studies were performed while the patients were in fasting and basal condition. The myasthenic patients had received no medication for 12 hours before, and none during the tests. Water (150 ml.) was administered orally every half hour for two hours prior to and during the period of urine collection to insure adequate urine flow. Urine was collected at intervals of approximately 20 minutes from an indwelling catheter, and a sample of venous blood was obtained during each collection period. Glomerular filtration rate was determined by inulin clearance in seven out of nine studies, using an intravenous infusion of 5 grams of inulin in 400 to 700 ml. of normal saline solution. The excretion of endogenous histidine was observed for one to four collection periods, following which 2 to 4 grams of l-histidine hydrochloride were added to the infusion and administered with the inulin over a period of 1½ to 2 hours. These amounts of histidine produced no symptoms. The initial rate of infusion was 10 ml. per minute for 8 minutes, followed by 4 to 8 ml. per minute. This produced rapidly rising concentrations of inulin and histidine in the serum, followed by sustained concentrations, which declined over a period of approximately 7 hours (inulin) and 1½ to 3½ hours (histidine) after the infusion was completed. The concentrations of inulin and of histidine at the mid point of each 20 minute urine collection period were obtained from the curves plotting logarithm of serum concentration against time.

The concentration of inulin was determined in the serum and urine by the method of Higashi and Peters (23). The concentrations of inulin and of histidine were the same in serum as in plasma, and in venous as in arterial blood, so that venous blood serum could be employed for the calculation of glomerular filtration rates. The infusion of inulin was found to have no appreciable effect on the renal clearance of either endogenous or injected histidine, and the infusion of histidine, in the amounts employed, did not alter the glomerular filtration rate, as measured by inulin clearance.

From the determined serum and urinary concentrations of histidine and inulin were calculated the clearance of inulin (the glomerular filtration rate), and the rates of glomerular filtration, urinary excretion, and renal tubular reabsorption of histidine. The rate at which histidine was filtered (tubular load, mg./min.) was calculated, assuming histidine to be freely diffusible, by multiplying the glomerular filtration rate (ml./min.) by the concentration of histidine in the serum (mg./ml.). Evidence for the complete diffusibility of serum histidine was provided by observations on the concentration of this compound in extracellular fluids, and in dialysates of serum. The rate of tubular reabsorption of histidine (mg./min.) was calculated by subtracting the observed rate of urinary excretion (mg./min.) from the calculated tubular load. The renal clearances of inulin and of histidine (ml./min.) were obtained by dividing the rates of urinary excretion (mg./min.) by the serum concentrations (mg./ml.).

The renal and extrarenal components in the disappearance of histidine from the extracellular fluid following intravenous infusion were determined. The rate of extrarenal disappearance of extracellular histidine (mg./min.) was calculated by subtracting the rate of renal excretion from the rate of disappearance of histidine from the extracellular fluid. The amount of histidine (mg.) in the extracellular fluid at any time was calculated from the product of the serum concentration of histidine (mg./ml.) and the volume of the extracellular compartment, expressed as the volume of distribution of inulin (ml.). The latter was

determined by dividing the amount of inulin (mg.) excreted in the urine during eighteen hours after cessation of the infusion by the serum concentration (mg./ml.) at the time of termination of the infusion (24). The duration of infusion required to insure completely uniform distribution of inulin throughout the extracellular fluid has been reported to be five hours in man, but equilibrium is approached asymptotically and is 85 per cent complete in less than half this time (24). The volume of distribution of inulin determined by this method approximated that calculated by applying the post-infusion slope method (usually employed for the estimation of mannitol space (25)) to the slope of the falling curve of serum inulin concentration plotted semi-logarithmically against time. This suggests that uniform distribution of inulin in the extracellular fluid was approached at the termination of the infusion.

The renal and extrarenal components in the disappearance of infused histidine were also compared by calculation of the volume of extracellular fluid nominally cleared of histidine per minute by renal and extrarenal means. The volume cleared per minute by both means (the total clearance, ml./min.) was determined by dividing the rate of disappearance of histidine from the extracellular fluid (mg./min.) by the serum concentration of histidine (mg./ml.). The extrarenal clearance was calculated by subtracting the renal clearance from the total clearance.

The three patients in whom studies of renal filtration and reabsorption of histidine were performed were on a constant daily diet during the entire period of observation before, during, and after the administration of ACTH or cortisone. The same food was offered each day, made up of 78 gm. protein, 50 gm. fat, and 205 gm. carbohydrate for patient A. F., and 79 gm. protein, 77 gm. fat, and 225 gm. carbohydrate for patients M. Bi. and P. A. The other patients studied received regular hospital diets. During the period of ACTH or cortisone administration all patients were limited in their daily dietary intake of sodium chloride to four grams, and they were given three grams of potassium chloride daily. The two myasthenic patients (M. Bi. and A. F.) received 105 and 240 mg. of prostigmine bromide orally each day during the entire period of observation.

The administration of ACTH or cortisone, in doses recorded in Table 3, resulted in definite clinical improvement in all the patients with allergic or "hypersensitive" disease, while the two patients with myasthenia gravis became slightly weaker during the period of administration. In all the patients there was a reduction in the number of circulating eosinophiles during hormone administration.

RESULTS

Blood Level and Renal Excretion of Histamine and Histidine in Nine Normal Subjects (Table I)

The concentration of histamine in the plasma was below the minimal detectable concentration by the method employed. The centrifuged red and white blood cells of only three subjects (D. G., G. J., and B. Z.) yielded detectable colorimetric reactions for histamine, equivalent to 0.35, 0.4, and 0.6 μg./ml. of histamine base. The effect of acid hydrolysis and incubation with histaminase, which destroyed most of the color producing substance, indicated that the colorimetric reaction obtained with hemolyzed blood cells could be attributed primarily to acetyl histamine, and that only a small part of the reaction was due to non-histamine substances. The latter were not identified, but were

neither histidine nor tyrosine. The average concentration of histidine in the serum (fasting) was 0.012 mg./ml.

TABLE I

Mean and Standard Deviation About Each Patient's Mean of Daily Fasting Serum Level of Histidine and of Daily Urinary Excretion of Histamine and Histidine in Nine Normal Subjects, Thirteen Patients with Allergic or "Hypersensitive" Diseases, and Two with Myasthenia Gravis

The "standard deviation" is noted even when the number of observations is limited in order to indicate the extent of day to day variation.

SUBJECT	SEX	AGE	DISEASE	DAYS OBSERVED	SERUM HISTIDINE mg./ml.		URINE HISTAMINE mg./24 hrs.		URINE HISTIDINE mg./24 hrs.	
					Av.	S.D.	Av.	S.D.	Av.	S.D.
I. B.	F	30	None	2	0.013	0.001	—	—	—	—
D. G.	M	32	None	3	0.014	0.002	11.5	4.8	49.5	6.4
G. J.	M	23	None	2	0.008	0.002	15.4	9.3	106.3	23.7
M. P.	M	59	None	6	0.016	—	4.3	3.4	33.5	41.4
G. R.	F	33	None	4	0.011	—	5.8	1.4	79.4	32.0
G. S.	F	24	None	2	0.013	0.003	—	—	—	—
B. S.	F	29	None	10	0.008	—	5.3	3.6	42.9	25.2
W. W.	M	68	None	4	0.010	—	6.2	5.2	116.8	23.2
B. Z.	F	25	None	3	0.011	0.004	4.6	0.2	44.7	1.3
Avg. of normal subjects					0.012	0.002	7.6	4.0	67.6	21.9
M. Gr.	M	58	Bronchial asthma	7	0.011	0.001	3.8	0.6	58.0	46.2
F. L.	F	55	Bronchial asthma	4	—	—	3.4	1.4	36.4	8.6
H. N.	M	68	Bronchial asthma	7	0.010	0.002	7.4	3.5	95.4	43.9
M. S.	F	27	Bronchial asthma	2	—	—	1.8	1.9	10.6	2.2
M. H.	F	48	Bronchial asthma	8	0.014	0.006	19.7	4.5	142.0	52.7
J. F.	M	30	Bronchial asthma	11	0.014	0.004	2.6	1.3	—	—
E. M.	M	36	Bronchial asthma	3	—	—	5.6	5.4	119.1	13.5
J. S.	M	42	Bronchial asthma	5	—	—	6.7	7.4	74.0	29.1
M. Be.	M	46	Bronchial asthma	12	0.016	0.002	5.2	2.8	—	—
Average of patients with bronchial asthma					0.013	0.003	6.2	3.2	76.5	28.0
M. Gi.	F	20	Lupus erythem.	2	0.008	0.002	4.3	0.7	48.2	10.7
H. F.	F	42	Lupus erythem.	3	0.008	—	2.1	0.5	0	0
R. G.	F	49	Rheum. arthr.	8	0.010	—	8.5	3.8	147.9	34.9
P. A.	M	41	Cold allergy	9	0.016	0.001	7.4	2.8	101.0	54.8
Avg. of 13 pts. with allergic or hypersen. dis.					0.012	0.002	5.8	2.7	75.7	26.9
M. Bi.	F	26	Myas. gr.	6	0.013	0.001	9.4	6.0	104.2	56.5
A. F.	F	32	Myas. gr.	18	0.011	0.003	3.6	4.0	98.0	29.7

The average daily urinary excretion of histamine was 8 mg./24 hrs. and of histidine 68 mg./24 hrs. There was a wide range in the excretion of both sub-

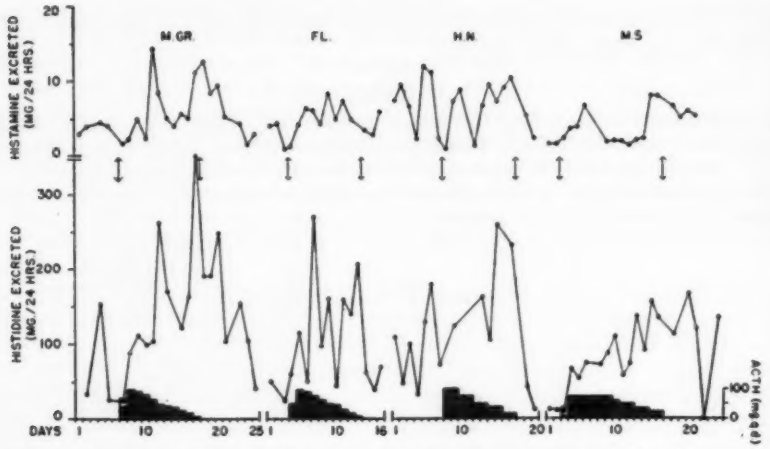


FIG. 1. Effect of ACTH administration on daily urinary excretion of histidine and histamine in patients M. Gr., F. L., H. N., and M. S. (bronchial asthma).

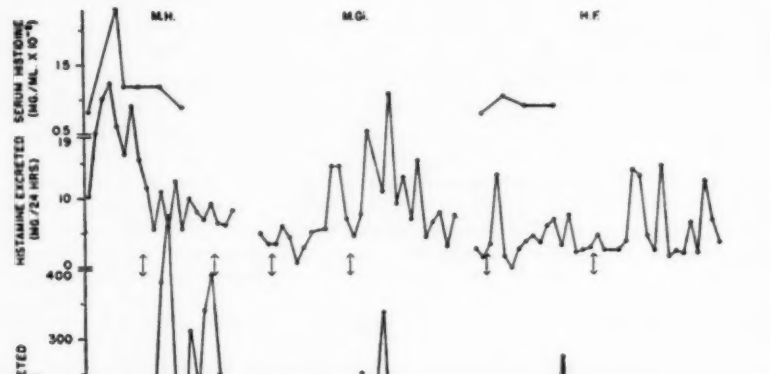


FIG. 2. Effect of ACTH administration on daily urinary excretion of histidine and histamine in patients M. H. (bronchial asthma) and M. Gi. and H. F. (lupus erythematosus), and on fasting serum concentration of histidine in M. H. and H. F.

stances, and considerable variaton in day to day excretion. Histamine was present in every specimen of urine obtained over a 24 hour period, and the colorimetric reaction was attributable primarily to acetyl histamine. Histidine was absent from the urine of one subject (M. P.) on four of six days. There appeared to be a rough correlation between the amount of histamine and of histidine excreted each day. The excretion of these compounds and the serum

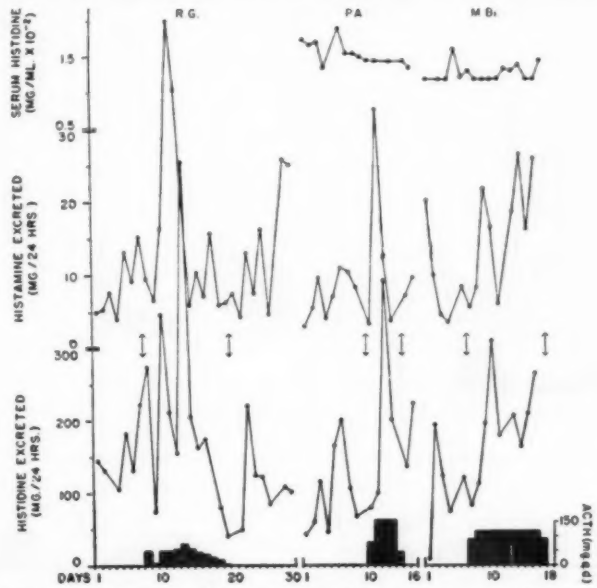


FIG. 3. Effect of ACTH administration on daily urinary excretion of histidine and histamine in patients R. G. (rheumatoid arthritis), P. A. (cold allergy), and M. Bi. (myasthenia gravis), and on fasting serum concentration of histidine in P. A. and M. Bi.

Patient P. A. received intravenous injections of histidine on days 9 and 14, and M. Bi. on days 5, 12, and 17. The excretion of histidine and histamine on these days has been omitted from the graph, as well as from the calculation of the average daily excretion in Tables 1 and 3.

concentration of histidine were not significantly different in male and female subjects.

Blood Level and Renal Excretion of Histamine and Histidine in Thirteen patients with Allergic or "Hypersensitive" Diseases, and in Two Patients with Myasthenia Gravis (Table I and Figs. 1–5)

The mean, range, and daily variations of serum histidine concentration and of daily urinary excretion of histamine and histidine in nine patients with bronchial asthma, one with cold allergy, two with lupus erythematosus, one with

rheumatoid arthritis, and two with myasthenia gravis were not significantly different from those of the normal subjects. The daily variations in excretion were not less in the three patients who were on constant diets than in the other patients or in the normal subjects. One patient with lupus erythematosus (H. F.) excreted no histidine during a five day period of observation.

As in the normal subjects, the concentration of histamine in the plasma was below the minimal detectable concentration by the method employed. The

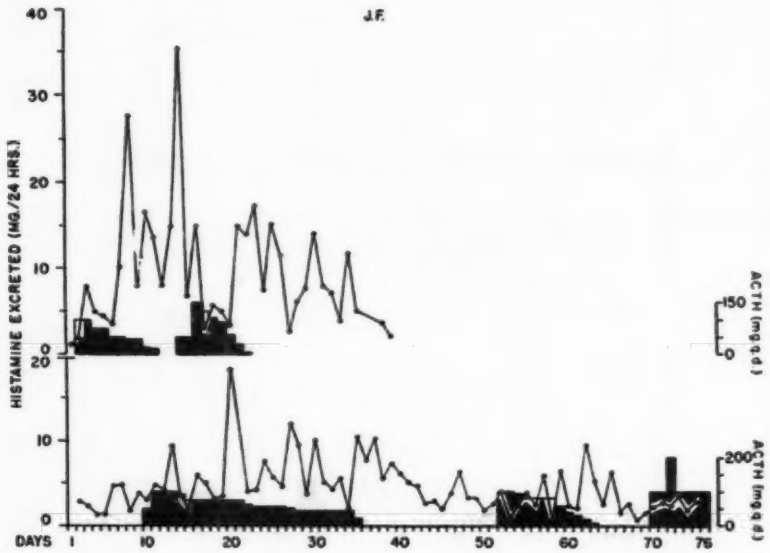


FIG. 4. Effect of five courses of ACTH on daily urinary excretion of histamine in patient J. F. (bronchial asthma). The time interval between the upper and lower graphs was 39 days.

centrifuged blood cells of only three patients, H. F. (lupus erythematosus) and M. H. and H. N. (bronchial asthma), yielded detectable colorimetric reactions for histamine, equivalent to 0.5, 1.5, and 2 μg./ml. These colorimetric reactions were attributable primarily to acetyl histamine.

The serum concentration of histidine and the urinary excretion of histamine and histidine bore no relation to the severity of bronchial asthma, to daily variations in asthmatic symptoms, or to the appearance of extensive urticarial lesions. The latter were observed to appear spontaneously in patient R. G., and were induced in patient P. A., along with joint pains, fever, and lymphadenopathy, by exposure to cold.

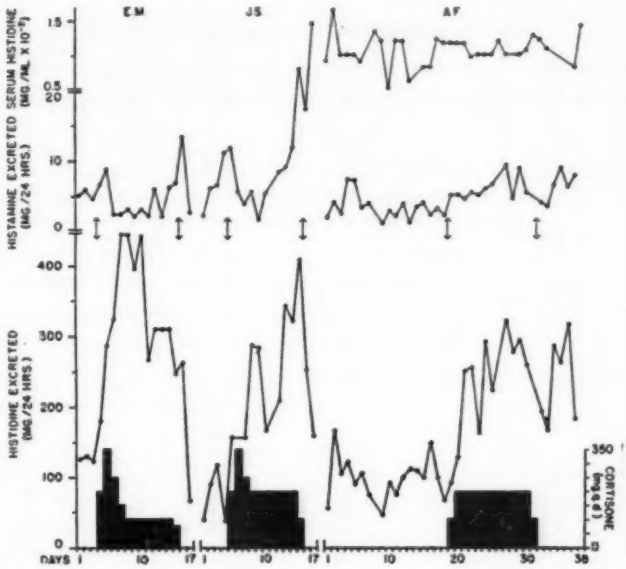


FIG. 5. Effect of cortisone on daily urinary excretion of histidine and histamine in patients E. M. and J. S. (bronchial asthma) and A. F. (myasthenia gravis), and on fasting serum concentration of histidine in A. F.

Patient A. F. received intravenous injections of histidine on days 8, 26, 31, and 38. The excretion of histidine and histamine on these days has been omitted from the graph, as well as from the calculation of the average daily excretion in Tables 1 and 3.

TABLE II

Comparison Between the Histidine Concentration in Serum and in Other Extracellular Fluids (Transudates and Exudates)

PT.	DISEASE	TRANSUDATE OR EXUDATE	CONCENTRATION OF					
			Histidine (mg. %)		N.P.N. (mg.%)		Total protein (Gm. %)	
			Serum	Trans.-Ex.	Serum	Trans.-Ex.	Serum	Trans.-Ex.
N. G.	Cardiac failure	Edema fluid	1.2	1.10	30	28	6.8	0.2
C. A.	Cardiac failure	Pleural fluid	1.0	0.94	32	23	7.2	1.0
C. A.	Lupus erythematosus	Pleural fluid	0.86	0.86	30	26	8.8	2.6
A. H.	Sarcoidosis	Pleural fluid	0.86	0.92	21	26	8.1	6.2
N. T.	Chronic glomerulonephritis, uremia	Edema fluid	2.15	2.10	160	162	6.7	0.6
A. J.	Chronic glomerulonephritis, uremia	Ascitic fluid	2.15	2.16	128	132	4.8	0.9

TABLE III

Summary of Effect of ACTH and Cortisone Administration on Serum Concentration of Histidine and Daily Urinary Excretion of Histamine and Histidine in Patients with Allergic or "Hypersensitive" Diseases or Myasthenia Gravis

PATIENT	DISEASE	ACTH		DAY OF ONSET CLIN. REMIS.	SERUM HISTIDINE (mg./ml., Av.)			URINE HISTAMINE (mg./24 hrs., Av.)			URINE HISTIDINE (mg./24 hrs., Av.)		
		mg. q.d.	Days		Before	During ACTH	After	Before	During ACTH	After	Before	During ACTH	After
M. Gr.	Bronchial asthma	55	11	1				4	6	6	58	147	146
F. L.	Bronchial asthma	53	10	3				3	6	4	36	130	56
H. N.	Bronchial asthma	57	10	2				7	7	6	95	189	28
M. S.	Bronchial asthma	55	14	2				2	4	6	11	87	107
M. H.	Bronchial asthma	57	10	2	0.014	0.010		20	9	8	142	288	172
J. F.	Bronchial asthma	64	21	1				3	11	8			
J. F.	Bronchial asthma	62	26	4	0.015	0.012		3	4	6			
M. Gi.	Lupus erythem.	49	19	3				4	9	7	48	148	102
H. F.	Lupus erythem.	56	15	3				2	5	6	0	85	48
R. G.	Rheum. arthr.	41	12	1	0.010	0.010		8	16	14	148	202	114
P. A.	Cold allergy	120	5	4	0.016	0.014	0.013	7	13	8	101	194	180
M. Bi.	Myas. gr.	112	11	None	0.013	0.013		9	16		104	192	
Average		65	14		0.014	0.013		6	9	7	74	166	106
		CORTISONE											
E. M.	Bronchial asthma	144	12	3				6	4	13	119	331	145
J. S.	Bronchial asthma	209	11	2				7	9	24	74	245	208
A. F.	Myas. gr.	186	13	None	0.011	0.011	0.012	4	6	6	98	214	253
Average		180	12					5	6	14	97	263	202

Mechanism of the Renal Excretion of Histidine

Diffusibility of histidine: Calculation of the glomerular filtration and renal tubular reabsorption rates of any constituent of the plasma is not possible

TABLE IV

Renal Excretion of Endogenous and Injected Histidine in Patient M. Bi. (age 26, 47 Kg., 1.46 sq. M.) Before and During the Administration of ACTH, and Renal Excretion, Reabsorption and Clearance Later in the Course of ACTH (120 mg. q.d.)

TIME min.	URINE FLOW ml./min.	INULIN CLEAR. ml./min.	HISTIDINE						
			Conc.		Filt'd.	Excre.	Reabsorb.		Clear. ml./min.
			Serum	Urine	mg./min.			%	
			mg./ml.						
A. Before ACTH: Infusion of 3 Gm. Histidine HCl Begun at Zero Time and Ending at 58 Minutes									
53–28	12.7		0.016	0		0			0
28–2	4.0		0.016	0.006		0.02			1.3
2–0–17	6.3		0.028	0.016		0.10			3.6
17–37	7.0		0.040	0.036		0.25			6.3
37–57	6.0		0.071	0.081		0.48			6.8
57–77	2.4		0.058	0.167		0.40			6.9
77–97	5.2		0.044	0.084		0.44			10.0
B. During ACTH (5th day): Infusion of 3 Gm. Histidine HCl Begun at Zero Time and Ending at 53 Minutes									
20–0	1.0		0.014	0.426		0.36			26.7
0–20	1.4		0.025	0.435		0.60			23.8
20–40	2.0		0.062	0.789		1.53			24.8
100–120	1.2		0.030	0.620		0.77			25.5
120–140	1.0		0.025	0.588		0.51			20.4
140–165	1.2		0.021	0.388		0.47			22.6
165–172	4.0		0.018	0.102		0.41			23.4
C. During ACTH (11th day): Infusion of 3 Gm. Histidine HCl Begun at Zero Time and Ending at 60 Minutes									
40–29	2.3	110	0.014	0.081	1.54	0.22	1.32	85.3	15.1
29–0–2	3.0	97	0.016	0	1.55	0	1.55	100.0	0
2–22	11.5	103	0.054	0.052	5.61	0.59	5.02	89.5	10.9
42–62	1.9	110	0.054	0.387	5.94	0.75	5.19	87.5	13.9
62–82	1.3	118	0.041	0.384	4.83	0.48	4.35	90.0	11.7
82–102	1.2	94	0.029	0.354	2.73	0.49	2.30	84.3	14.8
102–122	2.7	130	0.025	0.252	3.25	0.69	2.56	78.9	27.6

unless it is known to be freely diffusible within the extracellular compartment, or diffusible in known proportion to the plasma concentration. A number of observations, while indirect, indicated that histidine is probably filtered freely

through the glomeruli and throughout the extracellular compartment. The concentration of histidine in transudates, such as edema and ascitic fluid of patients in cardiac or renal failure, and of exudates, was approximately the same as in the serum, even when the serum concentration was elevated, as in uremia (Table II). Histidine, both endogenous and added, was freely diffusible from serum across collodion membranes. The dialysis against running tap water for 16 hours of serum containing 0.008 mg./ml. endogenous histidine, or en-

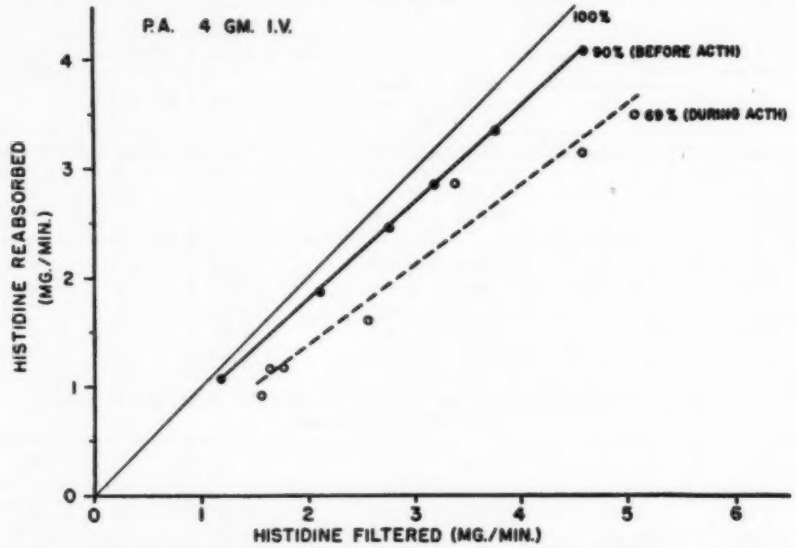


FIG. 6. Renal tubular reabsorption of endogenous and injected histidine in patient P. A. (cold allergy) before ACTH and on the fifth day of ACTH administration (160 mg.q.d.). The lowest filtration rate on each day is that of endogenous histidine, while higher rates are the result of the intravenous infusion of four grams of histidine hydrochloride. The line drawn at 45° represents theoretically complete reabsorption. The difference between this line and the recorded rate of reabsorption is the rate of excretion.

dogenous plus added histidine in concentration of 0.02 mg./ml., resulted in the disappearance of all detectable histidine.

Mechanism of renal excretion of endogenous histidine: In the patients whose renal clearance of histidine was studied, endogenous histidine was filtered at an average rate of 1.27 mg./min. and excreted at an average rate of 0.07 mg./min. (Tables IVa, Va, VIa, VII, Figs. 6, 7). Ninety to 100 per cent (average 94 per cent) of endogenous histidine filtered during each clearance period was reabsorbed. The rate of tubular reabsorption of endogenous histidine was not constant; complete reabsorption occurred at times, but there was no evidence of a critical serum concentration or tubular load below which reabsorp-

tion was always complete. The rates of excretion and reabsorption did not appear to be influenced by urine flow, within the limits of flow that were observed. The average renal clearance of endogenous histidine (ml. of plasma cleared per minute) was 5.5 ml./min.

Mechanism of renal excretion of injected histidine: As the serum concentration of histidine was increased progressively, thereby increasing the tubular load, the reabsorption rate increased almost proportionately (Tables Va, VIa, VII,

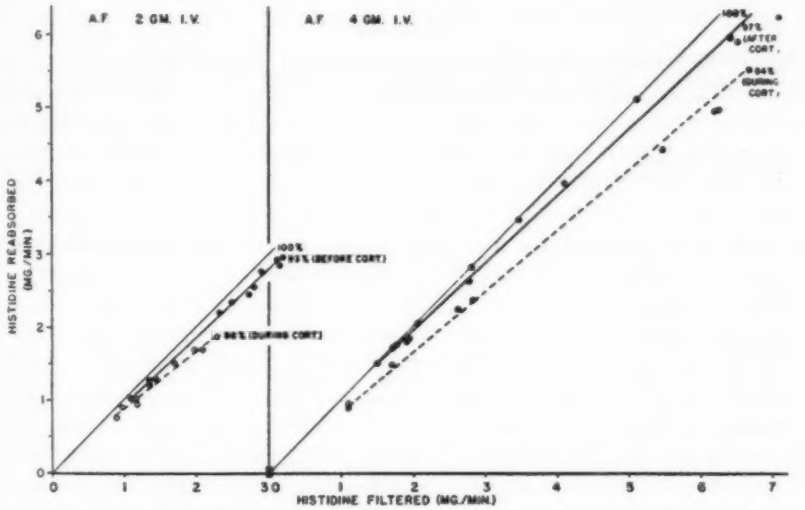


FIG. 7. Renal tubular reabsorption of endogenous and injected histidine in patient A. F. (myasthenia gravis) before and on the eighth day of cortisone administration (left), and on the thirteenth day of cortisone and seven days after cessation (right).

The lowest filtration rate on each day is that of endogenous histidine, while higher rates are the result of the intravenous infusion of two grams (left) or four grams (right) of histidine hydrochloride. The line drawn at 45° represents theoretically complete reabsorption. The difference between this line and the recorded rate of reabsorption is the rate of excretion.

Figs. 6, 7). Eighty nine to 95 per cent (average 92 per cent) of filtered histidine was reabsorbed at elevated loads. The almost constant ratio between filtration and reabsorption rates at all tubular loads indicates that a maximum rate of reabsorption was not reached at the highest tubular loads that were studied.

Fate of intravenously injected histidine: The intravenous injection of 2 to 4 grams of histidine hydrochloride by slow infusion resulted in a two to six fold increase in the serum level. The infused histidine was removed from the serum, and presumably from the extracellular fluid, over a period of 1½ to 3½ hours (Fig. 8). Only 2 per cent of the injected histidine appeared in the urine before

the serum concentration returned to its pre-injection level, indicating that the removal of histidine from the extracellular fluid was almost entirely owing to causes other than renal excretion. The rates of extrarenal disappearance (Fig. 9) and clearance (Fig. 10) of histidine following intravenous infusion were 10 to 30 times the rates of renal loss and clearance. The differences were greatest at elevated serum concentrations, the extrarenal loss and clearance increasing much more at elevated serum concentrations than did the renal.

TABLE V

Renal Excretion, Reabsorption, and Clearance of Endogenous and Injected Histidine in Patient P. A. (Age 41, 50 Kg., 1.51 sq. M.) Before and During the Administration of ACTH (160 mg. q.d.)

TIME min.	URINE FLOW ml./min.	INULIN CLEAR. ml./min.	HISTIDINE						
			Conc.		Filt'd.	Excre.	Reabsorb.		Clear. ml./min.
			Serum	Urine	mg./min.			%	
			mg./ml.						
A. Before ACTH: Infusion of 4 Gm. Histidine HCl Begun at Zero Time and Ending at 128 Minutes									
42–12	8.0	90	0.013	0.018	1.17	0.09	1.08	92.5	6.9
12–0–30	14.3	91	0.023	0.015	2.10	0.21	1.89	90.0	9.1
30–68	7.4	84	0.045	0.054	3.76	0.40	3.36	89.4	8.9
68–106	8.2	87	0.053	0.061	4.59	0.50	4.09	89.1	9.4
130–154	9.6	80	0.040	0.034	3.19	0.32	2.87	90.0	8.0
154–188	9.4	109	0.025	0.030	2.75	0.27	2.48	90.2	10.6
B. During ACTH (Day 5): Infusion of 4 Gm. Histidine HCl Begun at Zero Time and Ending at 112 Minutes									
39–9	0.9	120	0.014	0.330	1.63	0.46	1.17	71.8	33.8
9–0–32	0.9	106	0.032	0.536	3.38	0.50	2.88	85.2	15.6
62–92	3.1	127	0.040	0.507	5.08	1.57	3.51	69.1	39.3
92–122	4.0	144	0.032	0.360	4.59	1.43	3.16	68.8	47.1
158–182	8.4	157	0.016	0.112	2.56	0.94	1.62	63.2	57.6
182–212	8.0	133	0.013	0.073	1.76	0.58	1.18	67.0	44.4
212–242	10.9	146	0.011	0.060	1.55	0.63	0.92	59.4	59.4

If loss of histidine from the extracellular fluid by other means than renal excretion had not occurred, the serum concentration following intravenous infusion would have been eight times the observed value. The rapidity of this extrarenal loss is reflected by comparison with inulin, which disappears from the extracellular fluid solely as a result of renal excretion. When four grams of histidine hydrochloride were infused simultaneously with five grams of inulin the highest serum concentration of histidine attained was less than a third that of inulin, and the serum concentration of histidine returned to its original level in half the time required for the disappearance of inulin, despite the more than ten-fold greater excretion rate of inulin.

Since the incubation of whole blood containing added histidine for several hours at 37°C resulted in only slight decrease in the plasma concentration of histidine, and no increase in the red blood cell content, the extrarenal disappearance of histidine would appear to be due to uptake by cells outside the blood stream.

Effect of ACTH and Cortisone Administration on the Blood Level and Urinary Excretion of Histamine and Histidine in Patients with Allergic or "Hypersensitive" Diseases, or with Myasthenia Gravis (Table III and Figs. 1–5)

Blood levels: During the administration of ACTH or cortisone the plasma histamine remained below the minimal detectable concentration. The blood cell histamine remained below the minimal detectable concentration in all but the three patients whose blood cells had yielded detectable colorimetric reactions for histamine prior to hormone administration, and in these there was no appreciable change. The fasting serum level of histidine was not significantly changed.

Urinary excretion of histamine: In ten of fourteen patients there was a transient increase in excretion during ACTH or cortisone, usually occurring early in the course of administration, or when the dose was progressively reduced. In five of these patients the increase was three fold, and was six times the standard deviation of day to day excretion prior to hormone administration, while in five others the increase was only slightly greater than the spontaneous day to day variation. One patient had a transient three fold increase in excretion during three courses of ACTH, but little change during the fourth and fifth courses. In three patients there was no significant change in excretion during ACTH or cortisone, and in one patient, who had the highest daily excretion in the control period, there was a decrease during ACTH. The average increase in daily excretion during ACTH was no greater than the day to day variation prior to hormone administration, and that during cortisone was actually less, reflecting the transient nature of the increases that occurred.

Urinary excretion of histidine: In each of thirteen patients there was a striking and fairly sustained increase, which began on the first to the third day of hormone administration. The average increase was 2.2 fold during ACTH and 2.7 fold during cortisone. This was three and one half and six times the average standard deviation of day to day excretion prior to hormone administration, while the peak increases were ten times the standard deviation. Excretion usually reached a peak on the third to the sixth day, and then usually fluctuated, though remaining above the control level during the entire period of administration, which was up to 26 days. Reduction in the daily dose was followed in most instances by some decline in excretion. Following cessation of ACTH or cortisone the daily excretion diminished gradually over a period of two to six days, and returned to or near the original level in most patients.

TABLE VI

Renal Excretion, Reabsorption, and Clearance of Endogenous and Injected Histidine in Patient A. F. (age 32, 50 Kg., 1.53 sq. M.) Before, During and After the Administration of Cortisone (200 mg. q.d.)

TIME min.	URINE FLOW ml./min.	INULIN CLEAR. ml./min.	HISTIDINE						
			Conc.		Filt'd.	Excre.	Reabsorb.		Clear. ml./min.
			Serum	Urine	mg./min.			%	
			mg./ml.						
A. Before Cortisone: Infusion of 2 Gm. Histidine HCl Begun at Zero Time and Ending at 101 Minutes									
65–50	4.1	104	0.013	0.027	1.35	0.11	1.24	92.0	8.5
50–35	4.1	110	0.013	0.038	1.43	0.15	1.28	89.6	11.5
35–20	1.5	90	0.012	0.072	1.08	0.04	1.04	96.3	3.3
20–5	3.3	104	0.013	0.027	1.35	0.09	1.26	93.4	6.9
10–25	6.0	86	0.027	0.018	2.32	0.11	2.21	95.2	4.1
25–40	5.2	100	0.028	0.045	2.80	0.23	2.57	91.8	8.2
55–70	4.1	88	0.033	0.034	2.90	0.14	2.76	95.2	4.3
70–85	4.2	94	0.034	0.057	3.20	0.24	2.96	92.5	7.0
100–115	6.8	96	0.032	0.029	3.12	0.20	2.92	93.5	6.2
115–130	11.3	96	0.028	0.024	2.73	0.27	2.46	90.1	9.5
130–145	3.7	89	0.028	0.036	2.49	0.13	2.36	94.8	4.6
145–160	9.8	112	0.028	0.029	3.14	0.28	2.86	91.2	10.0
B. During Cortisone (8th day): Infusion of 2 Gm. Histidine HCl Begun at Zero Time and Ending at 126 Minutes									
57–37	2.5	89	0.010	0.041	0.89	0.13	0.76	88.5	13.0
37–17	2.5	96	0.010	0.024	0.96	0.06	0.90	93.6	6.0
17–0–3	4.4	89	0.013	0.030	1.16	0.13	1.03	88.8	10.0
3–23	6.9	57	0.020	0.034	1.18	0.24	0.94	79.9	11.7
68–83	3.6	85	0.024	0.102	2.08	0.37	1.70	81.9	15.1
83–103	9.1	81	0.024	0.030	1.97	0.27	1.70	86.2	11.0
123–143	5.6	92	0.024	0.066	2.26	0.37	1.89	83.7	15.1
143–163	1.3	75	0.022	0.131	1.70	0.20	1.50	87.8	8.9
C. During Cortisone (13th day): Infusion of 4 Gm. Histidine HCl Begun at Zero Time and Ending at 81 Minutes									
45–25	8.0	85	0.012	0.024	1.09	0.19	0.90	82.5	16.4
25–5	6.0	85	0.012	0.030	1.09	0.18	0.91	83.4	15.5
35–55	6.5	86	0.072	0.194	6.21	1.25	4.96	79.9	17.3
55–75	10.0	80	0.078	0.128	6.25	1.28	4.97	79.4	16.4
75–95	5.3	105	0.064	0.219	6.68	1.16	5.52	82.6	18.2
95–115	4.9	108	0.050	0.091	5.47	1.04	4.43	81.2	20.6
115–135	5.0	74	0.038	0.096	2.83	0.46	2.37	80.2	12.1
135–155	4.0	80	0.033	0.042	2.63	0.38	2.25	85.6	11.6
155–175	6.0	59	0.029	0.015	1.73	0.25	1.48	85.6	8.5
175–195	7.0	74	0.026	0.032	1.95	0.10	1.85	94.7	3.7
195–215	3.8	77	0.025	0.168	1.93	0.12	1.81	93.6	4.7

D. After Cortisone (7 days): Infusion of 4 Gm. Histidine HCl Begun at Zero Time and Ending at 107 Minutes

79–59	3.4	120	0.015	0	1.75	0	1.75	100.0	0
59–29	10.8	100	0.015	0	1.50	0	1.50	100.0	0
29–2	5.6	120	0.015	0	1.75	0	1.75	100.0	0
2–0–16	1.0	158	0.026	0.127	4.10	0.13	3.97	96.8	5.0
16–36	4.6	125	0.058	0.176	7.25	1.01	6.24	86.0	17.3
61–81	6.0	125	0.052	0.100	6.44	0.48	5.96	92.5	9.3
81–101	3.4	109	0.056	0.180	6.52	0.62	5.90	89.9	10.9
121–141	12.5	138	0.037	0	5.12	0	5.12	100.0	0
141–161	5.7	111	0.025	0.032	2.77	0.13	2.64	95.3	5.2
161–181	5.8	127	0.022	0	2.84	0	2.84	100.0	0
181–201	11.8	158	0.022	0	3.48	0	3.48	100.0	0
201–221	6.0	99	0.021	0	2.06	0	2.06	100.0	0

In some patients the peaks of increased histidine excretion were accompanied by increases in histamine excretion, occurring on the same day, the day after, or, less often, the day before (Figs. 1 and 3).

The effect of adrenal cortical stimulation or substitution on histamine and histidine excretion was essentially the same in the patients with myasthenia gravis, who showed no clinical improvement, as in the patients with allergic or "hypersensitive" diseases, who were improved.

Renal clearance of endogenous histidine (Tables IV–VII and Figs. 6–7): The daily administration of 120 to 160 mg. of ACTH or 200 mg. of cortisone resulted in a three fold increase in the average rate of urinary excretion and renal clearance of endogenous histidine, attributable primarily to decreased tubular reabsorption. Seventy-two to 93 per cent (average 82 per cent) of filtered endogenous histidine was reabsorbed, compared to an average of 94 per cent prior to hormone administration. An additional, but less important, factor that played a part in the increased excretion of histidine during ACTH in at least one patient (P. A.) was an increase in glomerular filtration rate (Table V). However, even in this patient, decreased tubular reabsorption played a greater role in the increased excretion, for while the rate of excretion of histidine was increased five fold during ACTH, the ratio of histidine to inulin clearance was increased three and a half fold. In patient A. F. the filtration rate actually decreased slightly during cortisone administration. The inulin clearance was not determined in patient M. Bi. prior to ACTH, but since this was 104 ml./min. during hormone administration, and the renal function by ordinary tests was normal, it is unlikely that the inulin clearance was significantly less prior to ACTH or that increased filtration rate played an important part in the increased excretion of histidine that occurred.

The rates of excretion and reabsorption of histidine were approximately the same in patient A. F. on the 8th and 13th days of cortisone administration,

while excretion was less in patient M. Bi. on the 11th than on the 5th day of ACTH. Seven days after the cessation of cortisone administration to patient A. F. the excretion of histidine was less, and reabsorption greater than prior to the administration of this compound.

TABLE VII

Average Renal Excretion, Reabsorption, and Clearance of Endogenous and Injected Histidine in Patients M. Bi., P. A., and A. F. Before, During and After the Administration of ACTH or Cortisone

The average figures for serum and urinary concentration of histidine, and rates of filtration, excretion, and reabsorption, following injected histidine are only roughly comparable, since the length of time of observation was not the same following each infusion (Tables 4–6).

PT.	ENDOGENOUS OR INJECTED HISTIDINE	DAY OF ACTH	NO. OF CLEAR. PERIODS	URINE FLOW ml./min.	INULIN CLEAR. ml./min.	HISTIDINE						
						Conc.		Filt'd.	Excre.	Reabsorb.		Clear. ml./min.
						Serum	Urine	mg./min.			%	
						mg./ml.						
M. Bi.	End.	−2	2	8.4		0.016	0.003		0.01			0.6
	End.	5	1	1.0		0.014	0.426		0.36			26.7
	End.	11	2	2.6	104	0.015	0.040	1.54	0.11	1.44	92.6	7.6
	Inj. 3 G.	−2	5	5.4		0.048	0.077		0.33			6.7
	Inj. 3 G.	5	6	1.8		0.030	0.470		0.72			23.4
	Inj. 3 G.	11	5	3.7	111	0.041	0.286	4.47	0.59	3.88	86.0	15.8
P. A.	End.	−1	1	8.0	90	0.013	0.018	1.17	0.09	1.08	92.5	6.9
	End.	5	1	0.9	120	0.014	0.330	1.63	0.46	1.17	71.8	33.8
	Inj. 4 G.	−1	5	9.8	90	0.037	0.039	3.28	0.34	2.94	89.8	9.2
	Inj. 4 G.	5	6	5.9	136	0.024	0.275	3.15	0.94	2.21	68.8	43.9
		DAY OF CORTISONE										
A. F.	End.	−11	4	3.2	102	0.013	0.041	1.30	0.10	1.20	92.8	7.6
	End.	8	3	3.1	91	0.011	0.032	1.00	0.11	0.93	90.3	9.7
	End.	13	2	7.0	85	0.012	0.027	1.09	0.18	0.90	83.0	16.0
	End.	7 After	3	6.6	113	0.015	0	1.66	0	1.66	100.0	0
	Inj. 2 G.	−11	8	6.4	95	0.030	0.034	2.84	0.20	2.64	93.0	6.7
	Inj. 2 G.	8	5	5.3	78	0.023	0.073	1.84	0.29	1.55	83.9	12.4
	Inj. 4 G.	13	9	5.8	82	0.046	0.109	3.96	0.67	3.29	84.7	12.6
	Inj. 4 G.	7 After	9	6.3	128	0.035	0.068	4.51	0.26	4.24	95.6	5.3

Renal clearance of injected histidine (Tables IV–VII and Figs. 6–7): The average rate of urinary excretion and renal clearance of histidine increased three fold at all serum concentrations and tubular loads. As in the case of endogenous histidine this was attributable primarily to decreased tubular reabsorption, 59 to 94 per cent (average 79 per cent) of filtered histidine being reabsorbed, compared to an average of 92 per cent prior to ACTH or cortisone.

The fraction of filtered histidine that was reabsorbed during ACTH or cortisone was only slightly less at elevated loads than at that offered by endogenous histidine, indicating that a maximum rate of reabsorption was not reached at any time.

As in the case of endogenous histidine clearance, the increase in glomerular filtration rate observed in one patient (P. A.) during ACTH administration contributed to the increased histidine excretion observed in this patient at all filtered loads, but to a lesser extent than the decreased tubular reabsorption.

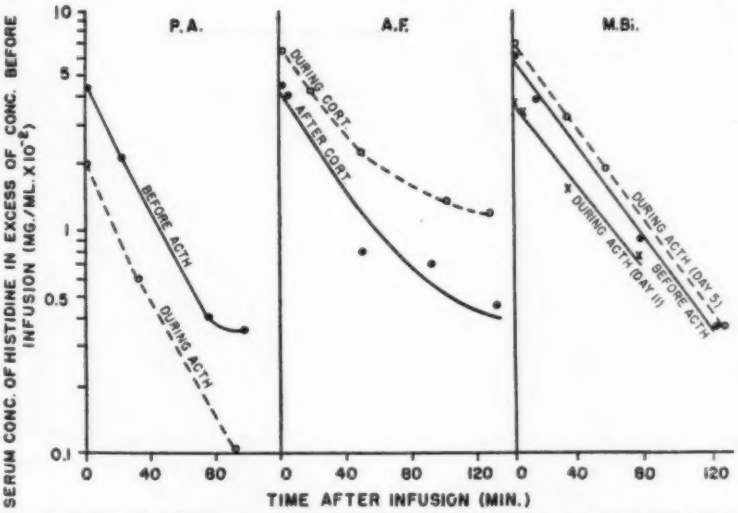


FIG. 8. Effect of ACTH and cortisone administration on the rate of fall of the serum concentration of histidine after the intravenous infusion of four grams of histidine hydrochloride (patients P. A. and A. F.) or three grams (patient M. Bi.). The pre-infusion concentration of histidine (Tables 4–6) has been subtracted from the observed concentration and the difference plotted on logarithmic scale.

In the other two patients alterations in filtration rate probably played little or no part in the increased histidine excretion at any filtered load. In patients M. Bi and A. F. alterations in the clearance of injected histidine later in the course of ACTH and cortisone administration, and following cessation of cortisone, paralleled the alterations in endogenous histidine clearance.

The ACTH preparation that was administered had some antidiuretic activity, which resulted in a diminution in daily urinary output, and in urine flow following intravenous infusion. The increased reabsorption of water did not appear to prevent the inhibitory action of ACTH on tubular reabsorption of histidine, so that the urinary concentration of histidine increased to an even greater degree during ACTH administration than did the rate of excretion.

The administration of cortisone, on the other hand, had no significant effect on urine flow.

Fate of intravenously injected histidine (*Figs. 8–10*): An average of 6 per cent of injected histidine appeared in the urine before the serum concentration returned to its pre-injection level, compared to 2 per cent prior to ACTH or cortisone. The serum concentrations attained during the infusion could not be compared, since the rates of infusion were not constant. The rate of disap-

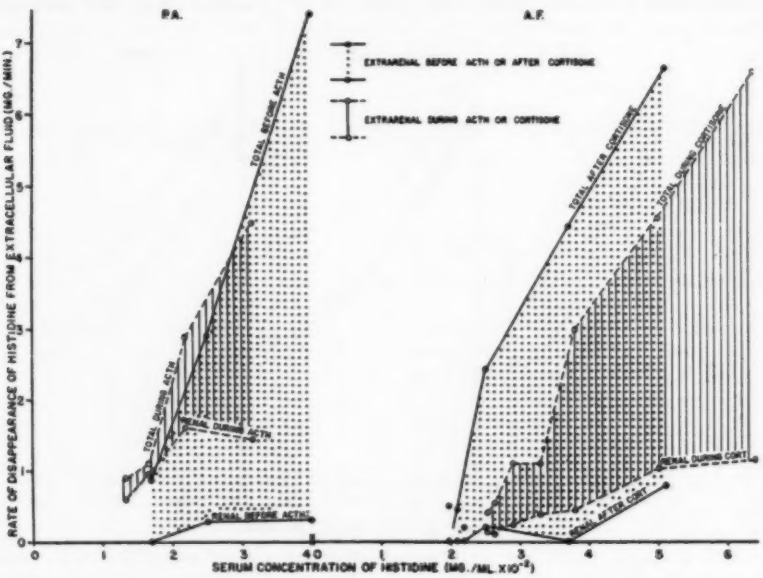


FIG. 9. Effect of ACTH and cortisone administration on the rates of total, renal, and extrarenal disappearance of histidine from the extracellular fluid following the intravenous infusion of four grams of histidine hydrochloride.

The volume of the extracellular fluid compartment (ml.) was:

Patient P. A.: *Before ACTH*, 8,500; *During ACTH* (*day 5*), 9,100.

A. F.: *Before Cortisone*, 7,000; *During Cortisone*, 6,600 (*day 8*), 6,800 (*Day 13, above*); *After Cortisone*, 7,100.

pearance of histidine from the serum and extracellular fluid was not appreciably altered in the two patients who received ACTH, and was decreased at all serum levels in the patient who received cortisone. Since the renal excretion and clearance of histidine were increased during hormone administration, while the rate of disappearance from the serum and extracellular fluid was unchanged or decreased, the extrarenal loss and clearance must have been reduced. The rates of extrarenal disappearance and clearance were calculated to have decreased by about one third during the administration of ACTH to

patient P. A., and by about one half during the administration of cortisone to patient A. F. The decrease was more striking at low than at elevated serum levels of histidine.

Since the calculation of the rate of disappearance of histidine from the extracellular compartment is dependent on the volume of this compartment, and since the extrarenal clearance varies with it, the decrease in extrarenal loss and clearance observed during ACTH and cortisone could have occurred as a result of decrease in this volume, or it could have been the result of a

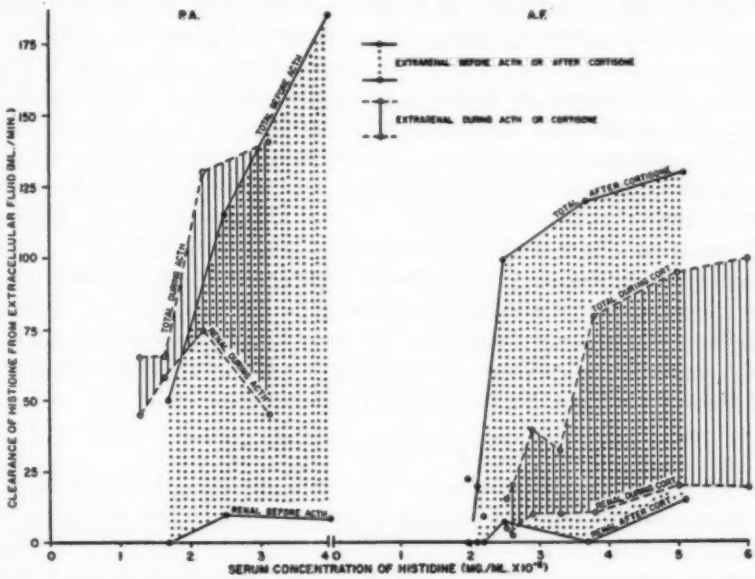


FIG. 10. Effect of ACTH and cortisone administration on the total, renal, and extrarenal clearance of extracellular histidine after the intravenous infusion of four grams of histidine hydrochloride.

The extracellular fluid compartment volumes are recorded in Fig. 9.

decrease in the rate of the extrarenal mechanisms by which histidine is removed from the extracellular fluid. The extracellular volume did diminish slightly during the administration of cortisone to patient A. F., but this could account for only a small part of the calculated decrease in extrarenal loss and clearance of histidine. The extracellular volume increased slightly during the administration of ACTH to patient P. A., indicating that the decrease in the rate of the extrarenal mechanisms by which histidine is removed was actually slightly more marked in this patient than the calculated decrease in extrarenal loss and clearance. The volume of the extracellular fluid compartment was not determined in patient M. Bi. prior to ACTH. If the volume determined on

the eleventh day of ACTH (8,000 cc.) is assumed to have been approximately the same as that present prior to ACTH and on the fifth day of administration, the extrarenal loss and clearance of histidine may be calculated to have decreased by about one sixth during the administration of ACTH to this patient.

DISCUSSION

Mechanism of Histidine Excretion

The very low concentration of histamine in the serum precluded study of the mechanism of renal excretion of this compound. Endogenous and injected histidine was found to be excreted by a mechanism involving tubular reabsorption which showed no limitation of transfer capacity in the range of tubular loads studied. The renal mechanism by which a small, but significant fraction of filtered histidine escapes reabsorption at tubular loads well below any possible saturation of the reabsorptive mechanism resembles the mechanism of urate (26), ascorbic acid (27), creatine (28), and inorganic phosphate (29) excretion. The excretion of histidine did not appear to be influenced by the rate of urine flow, indicating that histidine is actively reabsorbed, in contrast to the passive reabsorption of urea (30). The tubular reabsorption of histidine observed in the patients studied was not as complete as that reported in the dog, where over 99 per cent reabsorption has been observed at normal and markedly elevated plasma levels (to 0.41 mg./ml.) (11).

Effect of ACTH and Cortisone

The transient increase in histamine excretion, the more striking increase in histidine excretion, and the decrease in tubular reabsorption and extrarenal uptake of histidine which followed ACTH or cortisone administration occurred to the same extent in patients with myasthenia gravis, whose disease was not improved by these hormones, as in patients with allergic or "hypersensitive" diseases which were improved. These effects therefore appear to be attributable to the ACTH and cortisone, rather than to improvement in the patients' clinical condition.

The increase in urinary excretion of histidine during ACTH or cortisone administration is attributable chiefly to decreased tubular reabsorption, which was observed at all tubular loads of histidine. Since there is no evidence that amino acids can be secreted by the renal tubules, the only other possible cause of increased excretion is augmented tubular load of histidine. This may result from an increase in the glomerular filtration rate, a decrease in the rate of extrarenal uptake of histidine, or an increase in the plasma concentration. A moderate increase in glomerular filtration rate was observed during ACTH administration in one patient (P. A.), and comparable increases have been reported to occur in some patients after doses of ACTH and cortisone similar to

those that were administered (31). Decrease in the rate of extrarenal uptake of histidine was also observed during ACTH and cortisone administration. Increase in the fasting serum concentration was not observed, but Stephens and his coworkers (8) have reported a slight increase during ACTH administration, as determined by biological methods, and have suggested that this might be the cause of the increase in urinary excretion. However, since the fraction of filtered histidine reabsorbed was almost the same at elevated as at endogenous filtered loads, a two to three fold increase in tubular load would be required to produce the observed increase in excretion, and neither the observed changes in glomerular filtration rate and extrarenal uptake of histidine, nor the reported change in fasting serum concentration, are of an order sufficient to produce such an increase in tubular load. Therefore, while increased glomerular filtration rate and decreased extrarenal uptake of histidine may account for some of the increase in histidine excretion observed during ACTH and cortisone administration, most of the increase is attributable to diminished tubular reabsorption.

The mechanism by which cortisone, and the corticoids secreted by the adrenals after stimulation by ACTH, may decrease the tubular reabsorption of histidine is not known. The filtration of increased amounts of certain other amino acids might play a part, since this has been found to decrease reabsorption of histidine (in the dog), presumably by competition for a common receptor in the tubular cells (32). However, the known influence of cortisone and ACTH on other renal functions makes it more likely that certain corticoids exert a direct influence on the renal tubular cells. The administration of ACTH and cortisone has been found to increase the urinary excretion of nitrogen, uric acid, creatinine, inorganic phosphate, potassium (33), and amino-acid nitrogen (34, 35), and to decrease the tubular reabsorption of endogenous urate and inorganic phosphate (31). Decreased tubular reabsorption of creatine has been observed in patients with hyperadrenocorticism (28).

The mechanism of the extrarenal uptake of histidine, apparently into cells other than those of the blood, and the mechanism by which ACTH and cortisone administration may diminish this uptake, are also unknown. The liver may be one of the sites of extrarenal uptake of histidine, since hepatectomy of experimental animals has been reported to be followed by a prompt increase in the plasma concentration of this compound (36). It is possible that the decreased renal tubular reabsorption and extrarenal uptake of histidine induced by ACTH and cortisone may be the result of a widespread decrease in cellular transport or utilization of histidine. However, the possibility cannot be excluded that the decreased extrarenal uptake may be due to a homeostatic mechanism compensatory to the increased urinary excretion, perhaps mediated by decreased utilization or increased production. The lack of any significant decrease in the fasting serum concentration of histidine during ACTH or cortisone

administration, despite the increased urinary excretion, suggests that a decrease in the extrarenal uptake of histidine may exist even at fasting serum concentrations, and that it may be of the same order at these concentrations as the increase in urinary excretion.

It is of interest that the excretion of endogenous and injected histidine was less, and reabsorption greater, in one patient (A. F.) after the cessation of cortisone administration than prior to cortisone. This may be related to the decrease in adrenal cortical function which may follow cortisone administration.

The increase in urinary excretion of histidine that occurs during most normal pregnancies (9, 10) is of approximately the same magnitude as that observed during ACTH or cortisone administration. Many explanations have been offered for the histidinuria of pregnancy, but the most convincing is that of Page, who demonstrated a decreased renal threshold for intravenously injected histidine during pregnancy (12). Although he did not determine filtration and reabsorption rates, Page suggested that the increased excretion might be due to decreased tubular reabsorption of histidine. In view of the increased excretion of urinary corticoids that occurs during pregnancy (37), it seems likely that the histidinuria of pregnancy may be due at least in part to the action of these corticoids in decreasing the tubular reabsorption of histidine.

The increased urinary excretion of nitrogen produced by adequate doses of ACTH and cortisone, and by conditions of "stress" associated with increased adrenal cortical activity, has been reported to be one to four grams a day (33), of which about 10 per cent is amino-acid nitrogen (34, 35). This increased urinary excretion of nitrogen, which frequently results in negative nitrogen balance, has been ascribed to increased protein catabolism (33), and to decreased anabolism (38). The increased urinary excretion of endogenous histidine which was observed, and the reported increase of uric acid (33), amount to about 0.06 gram of nitrogen a day in each case. This increase in nitrogen excretion, comprising a tenth or less of the total increase in nitrogen excretion reported after ACTH or cortisone, is largely attributable to the effect of these hormones on renal tubular reabsorption. It is possible that the increased excretion during ACTH and cortisone of other nitrogenous compounds, such as amino acids other than histidine, and creatine, may also be largely the result of decreased tubular reabsorption, perhaps augmented by increased glomerular filtration. To this extent, part of the increased nitrogen excretion and negative nitrogen balance produced by these hormones and by "stress" may be renal in origin, and part of the increased protein catabolism attributed to these compounds may be a consequence, rather than a cause, of the increased urinary excretion of nitrogenous compounds. However, extrarenal factors probably play some part in the increased nitrogen excretion, since, in the case of histidine, there is evidence of a concomitant decrease in extrarenal uptake. In addition, Engel (39) has demonstrated an increase in the rate of accumulation of urea in the blood of nephrectomized rats following ACTH, cortisone,

and non-specific "stress." It remains for further study to determine the identity of nitrogenous compounds other than histidine, uric acid, and creatine whose transport across the renal tubules may be diminished by adrenal cortical hormones, the precise role of this diminished tubular transport in the increased nitrogen excretion and negative nitrogen balance induced by these hormones, the effect of adrenal cortical hormones on the transport of nitrogenous compounds across cell membranes other than those of the renal tubules and on the utilization of these compounds, and the relationship of such effects to the ability of these hormones to lessen tissue reactivity to various stimuli.

SUMMARY

1. The serum concentration of histidine and the daily urinary excretion of histamine and histidine were approximately the same in patients with allergic or "hypersensitive" diseases (including bronchial asthma, cold allergy, lupus erythematosus, and rheumatoid arthritis) as in normal subjects.

2. Endogenous and injected histidine was excreted by a mechanism involving tubular reabsorption which showed no limitation of transfer capacity in the range of tubular loads studied. Only 2 per cent of injected histidine was excreted in the urine. The remaining 98 per cent was removed from the blood stream within 1½ to 3½ hours.

3. The administration of ACTH or cortisone to patients with allergic or "hypersensitive" diseases or myasthenia gravis produced a transient increase in the urinary excretion of histamine, and a more striking increase in the urinary excretion of histidine. There was no significant alteration in the serum concentration of histidine or in the detectable blood cell content of histamine.

4. The increased excretion of histidine produced by ACTH or cortisone was primarily renal in origin, and attributable chiefly to a decrease in tubular reabsorption. In at least one patient, increased glomerular filtration played a lesser role.

5. The extrarenal uptake of histidine from the extracellular fluid was reduced during the administration of ACTH or cortisone. The diminished extrarenal and tubular transport of histidine induced by these hormones may possibly reflect a widespread decrease in the cellular transport or utilization of this compound.

6. It is suggested that part of the increased nitrogen excretion and negative nitrogen balance produced by ACTH and cortisone may be renal in origin.

The author is indebted to Dr. A. McGehee Harvey for his advice and encouragement, and to Drs. Kenneth L. Zierler and Elliott V. Newman for reading the manuscript of this paper and providing valuable suggestions. Drs. Philip F. Wagley and Lawrence E. Shulman kindly permitted the study of their patient, P. A.

REFERENCES

1. Code, C. F.: The source in blood of the histamine-like constituent. J. Physiol., 1937, 90, 349–364.

2. Rose, B.: Role of histamine in anaphylaxis and allergy. Amer. J. Med., 1947, **3**, 545-559.
3. Anrep, G. V., Ayadi, M. S., Barsoum, G. S., Smith, J. R., and Talaat, M. M.: The excretion of histamine in urine. J. Physiol., 1944, **103**, 155–174.
4. Rose, B.: Studies of the effect of ACTH on eosinophilia and bronchial asthma. Proc. First Clinical ACTH Conference, The Blakiston Co., Phila., 1950, 491–493.
5. Rose, B., Pare, J. A. P., Pump, K., and Stanford, R. L.: Preliminary report on ACTH in asthma. Canad. Med. Assn. J., 1950, **62**, 6–9.
6. Rose, B., Pare, J. A. P., Pump, K., Stanford, R. L., Mackenzie, K. R., and Venning, E. H.: Observations on the metabolic changes resulting from the administration of ACTH to patients with asthma and allied conditions. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 519–527.
7. Borden, A. L., Wallraff, E. B., Brodie, E. C., Holbrook, W. P., Hill, D. F., Stephens, C. A. L., Jr., Kent, L. J., and Kemmerer, A. R.: Plasma levels of free amino acids in normal subjects compared with patients with rheumatoid arthritis. Proc. Soc. Exp. Biol. and Med., 1950, **75**, 28–30.
8. Stephens, C. A. L., Jr., Wallraff, E. B., Borden, A. L., Brodie, E. C., Holbrook, W. P., Paul, W., Hill, D. F., Kent, L. J., Kemmerer, A. R., and Arthur, R.: Apparent free histidine plasma and urine values in rheumatoid arthritis treated with cortisone and ACTH. Proc. Soc. Exp. Biol. and Med., 1950, **74**, 275–279.
9. Langley, W. D.: Urinary histidine. Determination in normal and in pregnancy urine. J. Biol. Chem., 1941, **137**, 255–265.
10. Chattaway, F. W.: The estimation of histidine in urine and its excretion by normal and pregnant women. Biochem. J., 1947, **41**, 226–230.
11. Wright, L. D., Russo, H. F., Skeggs, H. R., Patch, E. A., and Beyer, K. H.: The renal clearance of essential amino acids; arginine, histidine, lysine, and methionine. Amer. J. Physiol., 1947, **149**, 130–134.
12. Page, E. W.: The mechanism of the histidinuria of pregnancy. Amer. J. Obst. and Gynecol., 1946, **51**, 553–559.
13. Holbrook, W. P., Hill, D. F., Stephens, C. A. L., Jr., and Kent, L. J.: Effects of ACTH and cortisone on rheumatoid arthritis. Arizona Med., 1950, **7**, 43.
14. Holbrook, W. P.: Discussion following reference 33. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 258–262.
15. Koessler, K. K., and Hanke, M. T.: Studies on proteinogenous amines. The excretion of imidazoles in the urine, and the faculty of normal intestinal bacteria to form toxic amines. J. Biol. Chem., 1924, **59**, 803–853.
16. References reviewed in Farmer, L.: Histamine sensitivity and anaphylactic response. Amer. J. Clin. Path., 1943, **13**, 365–367.
17. Rose, B., and Browne, J. S. L.: The effect of adrenalectomy on the histamine content of the tissues of the rat. Amer. J. Physiol., 1941, **131**, 589–594.
18. Thorn, G. W., Forsham, P. H., Frawley, T. F., Hill, S. R., Jr., Roche, M., Staehelin, D., and Wilson, D. L.: The clinical usefulness of ACTH and cortisone. New Eng. J. Med., 1950, **242**, 783–793, 824–834, 865–872.
19. Rosenthal, S. M., and Tabor, H.: An improved colorimetric method for the estimation of histamine. J. Pharmacol. and Exp. Therap., 1948, **92**, 425–431.
20. Knoop, F.: Eine farbenreaktion des histidins. Hofmeister's Bietr. Chem. Physiol. und Pathol., 1908, **11**, 356.
21. Pauly, H.: Über die konstitution des histidins. Zt. f. physiol. Chem., 1904, **42**, 508–518.
22. Edlbacher, S., Bauer, H., Staehelin, D., and Zeller, A.: Die colorimetrische und enzymatische bestimmung des histidins. Zt. f. physiol. Chem., 1941, **270**, 158–164.

23. Higashi, A., and Peters, L.: A rapid colorimetric method for the determination of inulin in plasma and urine. J. Lab. and Clin. Med., 1950, **35**, 475–482.
24. Schwartz, I. L., Schachter, D., and Freinkel, N.: The measurement of extracellular fluid in man by means of a constant infusion technique. J. Clin. Invest., 1949, **28**, 1117–1125.
25. Newman, E. V., Bordley, J., III, and Winternitz, J.: The interrelationships of glomerular filtration rate (mannitol clearance), extracellular fluid volume, surface area of the body, and plasma concentration of mannitol. Bull. Johns Hopkins Hosp., 1944, **75**, 253–268.
26. Berliner, R. W., Hilton, J. G., Yu, T. F., and Kennedy, T. J., Jr.: The renal mechanism for urate excretion in man. J. Clin. Invest., 1950, **29**, 396–401.
27. Ralli, E. P., Friedman, G. J., and Rubin, S. H.: The mechanism of excretion of vitamin C by the human kidney. J. Clin. Invest., 1938, **17**, 765–770.
28. Zierler, K. L., Folk, B. P., Magladery, J. W., and Lilienthal, J. L., Jr.: On creatinuria in man. The roles of the renal tubule and of muscle mass. Bull. Johns Hopkins Hosp., 1949, **85**, 370–395.
29. Schiess, W. A., Ayer, J. L., Lotspeich, W. D., and Pitts, R. F.: The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. J. Clin. Invest., 1948, **27**, 57–64.
30. Chasis, H., and Smith, H. W.: The excretion of urea in normal man and in subjects with glomerulonephritis. J. Clin. Invest., 1938, **17**, 347–358.
31. Ingbar, S. H., Kass, E. H., Burnett, C. H., Relman, A. S., Burrows., B. A., and Sisson, J. H.: The effects of ACTH on the renal tubular transport of uric acid, phosphorus, and electrolytes in patients with normal renal and adrenal function. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 130–137.
32. Beyer, K. H., Wright, L. D., Skeggs, H. R., Russo, H. R., and Shaner, G. A.: Renal clearance of essential amino acids: their competition for reabsorption by the renal tubules. Amer. J. Physiol., 1947, **151**, 202–210.
33. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F.: Observations on the physiological effects of cortisone and ACTH in man. Arch. Int. Med. 1950, **85**, 199–258.
34. Bergenstal, D. M., Landau, R. L., Kirsner, J., and Lugibihl, K.: Effects of ACTH and related hormones on amino-acid metabolism. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 250–257.
35. Bardawill, C. J., Gornall, A. G., Nishikawara, M., and Wightman, K. J. R.: Studies on the behavior of urinary amino nitrogen, serum alkaline phosphatase, and pseudo cholinesterase activity during ACTH therapy. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 264–272.
36. Flock, E. V., Mann, F. C., and Bollman, J. L.: Free amino acids in plasma and muscle following total removal of the liver. J. Biol. Chem., 1951, **192**, 293–300.
37. Venning, E. H.: Adrenal function in pregnancy. Endocrinology, 1946, **39**, 203–220.
38. Albright, F.: Cushing's syndrome, its pathological physiology, its relationship to adreno-genital syndrome, and its connection with problems of reaction of body to injurious agents ("alarm reaction" of Selye). Harvey Lectures, 1943, **38**, 123–186.
39. Engel, F. L.: Comparative effects of ACTH and stress in nitrogen metabolism. Proc. Second Clinical ACTH Conference, The Blakiston Co., Phila., 1951, **1**, 235–241.

FAILURE OF PODOPHYLLIN IN THE TREATMENT OF CUTANEOUS CARCINOMA

MAURICE SULLIVAN, LAURENCE ZELL AND HUGH McCULLOCH, JR.

Department of Medicine (Division of Dermatology), The Johns Hopkins University School of Medicine and Hospital

Received for publication March 18, 1952

The purpose of this communication is to report recurrences of cutaneous carcinomas which had previouly undergone involution following treatment with resin of podophyllin (1).

The clinical material consists of the cases described in the preliminary report (1), plus tumors of 7 patients treated soon after. In one group of patients (Table I) podophyllin was applied and the tumors were observed until they healed. Post treatment biopsies were taken when signs of recurrence were noted. In another group the treated tumors were excised 3, 4, 5, 8, 11, 13, 14, 30, and 32 days after treatment to provide histologic material.

METHOD OF TREATMENT

Podophyllin, U.S.P. was applied topically in the form of a 25 per cent suspension in mineral oil, 20 per cent suspension in tincture of benzoin, or a 20 per cent solution in alcohol. The three preparations were equally effective but the latter two were more conveniently applied. Basal and prickle carcinomas selected for treatment were ulcerated and pretreatment biopsies further enlarged the ulcers. Applications were made to the tumor and a 1 cm. wide circumjacent band of normal skin and the treated area was not cleansed until podophyllin had been in contact for 24 hours. The macerated and necrotic surface tissue was wiped away with gauze before subsequent applications were made.

PATTERN OF CLINICAL "CURE"

Four days after the first application, maximum therapeutically induced ulceration was manifested by a central crater surrounded by a deeper clear cut peripheral moat and an erythematous, edematous halo of circumjacent normal skin. In three to four days the peripheral band of redness and swelling subsided unless additional applications were made. In seven to ten days there was a leveling of the base of the ulcer unless additional applications were made; epithelialization took place in the variable periods noted in Table I. Healed scars were pliant and resembled the slightly atrophic scars seen after successful roentgen ray treatment of small carcinomas; the resemblance was further suggested by the presence of telangiectasia in some cases. In sections of circumjacent normal skin taken during the first week after treatment there was swelling of the epidermal cells in the most superficial layers and an in-

Work supported by a Grant-in-Aid from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

crease in keratohyalin granules, accentuation of cell outlines with apparent overlapping of the cell margins, pyknosis and perinuclear vacuolation and the cytoplasm of some cells stained intensely eosinophilic. In and above the basal layer were many cells with distorted chromatin patterns representative of ar-

TABLE I

Results of a group of basal cell carcinomas (BCC), prickle cell carcinomas (PCC), and superficial intraepidermal epitheliomas (SIE) treated with podophyllin by various schedules

CASE NO.	DIAG.	LOCATION	SIZE IN CM.	NO. Rx	NO. DAYS	HEALING TIME IN DAYS	RECURRENCE AFTER COMPLETION OF TREATMENT IN:	NO RECURRENCE AFTER COMPLETION OF TREATMENT IN:
1	BCC	Nose	2.	15	62	70	22 months	
2	BCC	Nose	.8	5	14	24	17 months	
3	BCC	R. cheek	.5	3	18	18	16 months	
4	BCC	L. cheek	2 x 2.5	21	21	35	12 months	
5	BCC	Nose	1 x 2	10	12	50	3 months	
6	BCC	Forehead	2 x 3	25	117	150	2 months	
7	BCC	R. retroauricular	1.5	16	16	36	7 weeks	
8	BCC	L. eyebrow	1.5	17	17	21	6 weeks	
9	BCC	L. scapular	2 x 3	4	8	29	6 weeks	
10	BCC	Nose	2.	12	90	90	1 month	
11	PCC	L. temple	5.	12	31	60	1 month	
			5.	11	39	75	2 months	
12	SIE	R. neck & scalp	8 x 14.	10	49	70	6 months	
13	SIE	Lumbar region	4 x 6.	48	190	200	1 month	
	SIE	Anterior chest	2.	17	64	80		1 year
	SIE	Anterior chest	2.	17	64	80		1 year
	SIE	Anterior chest	2.	17	64	80		1 year
	SIE	Anterior chest	3.	17	64	80		1 year
	SIE	Upper back	3.	17	64	80		1 year
14	SIE	R. scapular	.8 x 1.2	16	90	100		3 years
	SIE	R. lumbar	2.	16	150	160		3 years
	SIE	L. occipital	1 x 2.	14	18	30		3 years
	SIE	L. post. neck	1.	14	18	30		2 years
	SIE	L. post. neck	1.	14	18	30		2 years
	SIE	L. post. neck	1.5	14	18	30		2 years
	SIE	L. post. neck	1.5	14	18	30		2 years
	SIE	L. shoulder	1.5	14	18	30		2 years
	SIE	L. shoulder	1.5	14	18	30		2 years
	SIE	L. shoulder	1.5	14	18	30		2 years
	SIE	L. shoulder	2.	14	18	30		2 years

rested mitotic figures in various stages of dispersion and fragmentation. These alterations were not observed in sections of normal skin taken one week after completion of treatment. In the superficial or uppermost layers of tumors there was cellular injury manifested by swelling, pyknosis, perinuclear fragmentation and cytoplasmic disintegration. Very few arrested mitoses (or

"podophyllin cells") were noted. Deeper masses of cells were undisturbed following a single application, but when necrotic material was wiped away and multiple applications were made, there was destruction of the lower layers of the tumor.

PATTERN OF RELAPSE

In the majority of relapses the first clinical sign was a nodule in or near the center of the healed scar. By contrast relapse of cutaneous carcinoma inadequately treated by electrodesiccation, cauterization, excision or roentgen therapy is usually manifested by a nodule or infiltration on the periphery of the previous tumor site. On microscopic examination masses of tumor cells were found below connective tissue which was interposed between the recurrent tumor cells and the overlying new epidermis. In case 8 a deep section was taken after completion of treatment and no tumor cells were seen. Seven weeks later when a small nodule appeared in the center of the scar the treated area was widely excised and there were many large masses of basal cells deep in the cutis.

RESULTS

Ten patients (Cases 1 to 10) with ten basal cell epitheliomas treated according to various schedules (Table I) healed in the periods noted; all recurred in 6 weeks to 22 months. One patient (Case 11) with a large prickle cell carcinoma exhibited healing after 12 treatments in 31 days. There was a relapse one month after cessation of treatment and on retreatment with 11 applications in 39 days there was again healing with complete epithelization followed by a second relapse 2 months later. Three patients with a total of 18 multiple superficial intraepidermal epitheliomas were treated. Two of the superficial lesions in 2 patients (Cases 12 and 13) were large, namely 8 x 14 cm. and 4 x 6 cm. Relapse occurred after apparent healing in 6 and 1 months respectively. The remaining 16 superficial intraepithelial epitheliomas were smaller, ranging in size from 1 to 3 cm. No recurrences 1 year later at the time of death were evident in one patient (Case 13). No recurrences in 3 years in 9 tumors were noted in Case 14.

DISCUSSION

Two types of action have been ascribed to podophyllin: 1) Arrest of mitoses; 2) direct cytotoxic injury (2–5). Involution of epidermal cells constituting condyloma acuminatum (3, 4) is probably accomplished by the latter, whereas, both types of action occur in normal skin circumjacent to treated condylomata and recovery occurs within a week. The clinical and microscopic effects in the superficial and the accessible portions of basal and prickle cell carcinoma are similar to those seen in condylomata; when directly exposed tumor cells are

destroyed. Deeper layers of tumor cells may be destroyed layer by layer provided the overlying macerated tissue is wiped away permitting access of the drug to the cells. Interposed connective tissue acts as a barrier and prevents contact with tumor cells below.

Other investigators (6,8) have reported "cures" of cutaneous carcinoma with podophyllin; we anticipate reports of recurrences. Sims and Pensky (9) were unable to produce involution of uneroded tumors.

CONCLUSIONS

1. Treatment of cutaneous basal and prickle cell carcinomas with podophyllin is dangerous and misleading as demonstrated by 100% recurrences of treated tumors in 6 weeks to 22 months. 2. The method should be used only as an experimental procedure for small intraepidermal epitheliomas, where the tumor cells are confined and apparently accessible to the direct destructive cytotoxic action of the resin.

REFERENCES

1. Sullivan, M.: Treatment of cutaneous carcinomas with podophyllin. Bull. Johns Hopkins Hosp. 1949, **85**: 200.
2. King, L. S. and Sullivan, M.: Similarity of podophyllin and colchicine effects and the use of these drugs in the treatment of condyloma acuminatum. Science. 1946, **104**: 244.
3. Sullivan, M. and King, L. S.: Effects of resin of podophyllum on normal skin, condylomata acuminata and verrucae vulgares. Arch. Dermat. and Syph. 1947, **56**: 30.
4. King, L. S. and Sullivan, M.: Effects of podophyllin and of colchicine on normal skin, on condylomata acuminata and on verruca vulgaris (Pathologic Observations). Arch. Pathology. 1947, **43**: 374.
5. King, L. S.: Effects of podophyllin on mouse skin. J. Nat. Cancer Institute. 1948, **8**: 215.
6. Smith, L. M. and Garrett, H. D.: Resin of podophyllum in the treatment of cancerous and precancerous treatment of the skin. Arch. Dermat. and Syph. 1947, **48**: 56.
7. Wyss-Chodat, F.: Nouvelle méthode de traitement des epithéliomas. La Podophyllinotherapie (Peltacure). Schweizerische medizinische Wochenscrift 1950, **80**: 647.
8. Wolf, C.: Infiltrating ulcerating basal-cell epithelioma treated with podophyllin. Arch. Dermat. and Syph. 1951, **64**: 245.
9. Sims, F. C. and Pensky, N.: Effects of podophyllin on basal cell epitheliomas. Arch. Dermat. and Syph. 1951, **64**: 142.

THE RELATION OF DOSAGE SCHEDULE TO THE THERAPEUTIC EFFICACY OF TERRAMYCIN[1]

LOUIS LASAGNA[2] AND C. G. ZUBROD

Department of Pharmacology and Experimental Therapeutics, and Department of Medicine, The Johns Hopkins University School of Medicine

Received for publication April 4, 1952

Terramycin was introduced early in 1950 as a new antibiotic with a wide range of antibacterial activity (1). The drug has enjoyed extensive use and has inspired several hundred reports in the medical literature both in this country and abroad (2). Therapeutic trials in humans antedated the accumulation of certain basic pharmacological data. One lacuna has been the absence of any information on the relation of dosage regimen to the efficiency of terramycin or similar antibiotics in experimental infections. There are scattered data in the literature (3, 4) on the relative efficacy of terramycin and aureomycin when given in a single dose or spread out over a number of days, but such data provide little basis for drawing conclusions because of the failure to maintain a roughly constant period of treatment. This paper deals with the results obtained in a study of the relationship of efficacy to dosage schedule in a pneumococcal infection in mice.

A. EFFECTIVENESS OF DIFFERENT DOSAGE REGIMENS

Methods. Carworth Farms mice (CF1 strain) were employed throughout the study. At the time of inoculation, the animals weighed between 15 and 21 grams. The infecting organisms were type I pneumococci, McGovern strain,[3] which were passed several times per week in mice to obtain and maintain maximal virulence. The cultures were kept at 4°C. between passages. Passage cultures were incubated at 37°C. in beef heart rabbit blood broth for 16 hours, at the end of which time a 10^{-1} subculture was made, again in beef heart blood broth. This subculture was incubated for a period of 6 hours, at the end of which time a 10^{-6} dilution was prepared in trypticase soy phosphate broth. One-half ml. of this dilution was found to contain from 500 to 2000 pneumococci, and was used as the inoculum, being injected intraperitoneally. This in-

[1] This investigation has been aided in part by a grant-in-aid from the United States Public Health Service.

[2] Sharp and Dohme Fellow in Pharmacology and Experimental Therapeutics.

[3] This strain of pneumococcus was obtained through the kindness of Dr. Leon H. Schmidt, Institute of Medical Research, Christ Hospital, Cincinnati, Ohio. It had been vacuum-dried in 1944 and preserved in that state until the time of use.

fecting dose produced bacteremia within an hour, and was uniformly fatal[4] within 12 to 26 hours.

The terramycin solution used was made by dissolving crystalline terramycin hydrochloride[5] in 0.1 N HCl, the latter being used because of the rather low solubility of terramycin in water (5). All values for amounts of terramycin in this paper refer to the base as such, and not the hydrochloride. Solutions were made up fresh each 24 hours, although there was no significant change in color or pH during this time. Injections of the drug were made into the hind limbs of the animals, with the first dose being administered 2½ hours after infection in all cases.

Several different dosage regimens were employed in this investigation. The duration of treatment was maintained constant at four days. The effect of varying the period between injections from 4 to 48 hours was studied for each of 6 different total doses. In addition, a few experiments were run to study the efficacy of a single dose schedule, i.e., the entire 4-day dose administered at one time, and will be referred to as "q.96 hours". It is realized that such a schedule cannot be strictly compared to regimens where drug is actually administered for 4 days.

The data were accumulated in five experiments. During the first three experiments, all of the dosage schedules for any particular total dose were run simultaneously. In the last two experiments, however, a few regimens were eliminated in order to concentrate the animals at certain points. The reproducibility of results in different experiments is shown in table 1.

All mice were observed for a period of two weeks after infection, although 96 per cent of the deaths occurred by the end of the sixth day. The heart blood of any mouse dying after the sixth day, (i.e., more than 48 hours after the cessation of therapy) was cultured. Such cultures invariably revealed the presence of type I pneumococci.

Results. The survival data obtained with the various dosage regimens employed are summarized in table 2. It is readily apparent that the interval between doses only affects therapeutic efficacy within an extremely narrow range in total dose. Thus, when the total dose for the four days is 100 mg. or more per kg., essentially all the mice are saved, whether the total 4-day course is given in 4-, 8-, 12-, 24- or 48-hourly doses. Similarly, the dosage regimen fails to in-

[4] One control mouse survived out of 100 injected. This mouse was reinfected at a later date with an inoculum of similar size and character and died within 18 hours.

[5] The terramycin hydrochloride used had been crystallized three times and represented the purest material available. All drug used came from a single lot, No. WBW 517361. The ampoules were kept in a desiccator after opening. We are greatly indebted to Dr. Gladys L. Hobby of Chas. Pfizer and Co., Brooklyn, New York, both for a generous supply of drug and for the above information on purity.

fluence survival at the 12.5 mg. per kg. level, where essentially all the animals die, regardless of dosage schedule.

At the 25 mg. per kg. level, there is definite evidence that the interval between doses is of importance, since the 4-hourly regimen is significantly more effective ($p = <0.01$) than any other regimen. At the 50 mg. per kg. level,

TABLE I

Reproducibility of Results in Successive Experiments with Terramycin in Treatment of Pneumococcal Infection of Mice

DOSAGE REGIMEN	DATE OF EXPERIMENT	NO. SURVIVORS/NO. MICE INFECTED		
		Total Dose of Terramycin in Mg./Kg.		
		25	50	100
12 Doses at 8 hr. Intervals	10/30/51		2/5	5/5
	11/4 /51	1/10	7/10	10/10
	11/12/51	0/20	18/20	10/10
	11/18/51	0/10	10/10	
24 Doses at 4 hr. Intervals	10/30/51		4/5	5/5
	11/4 /51	2/10	8/10	10/10
	11/12/51	4/20	20/20	10/10
	11/115/8	4/10	10/10	

TABLE II

The Influence of the Dosage Regimen on the Therapeutic Activity of Terramycin in Pneumococcal Infection of Mice

NO. OF DOSES AND DOSAGE INTERVAL	NO. SURVIVORS/NO. MICE INFECTED					
	TOTAL DOSE OF TERRAMYCIN (MG./KG.)					
	400	200	100	50	25	12.5
Single Dose			10/10	15/30	1/67	0/57
2 Doses at 48 hr. Intervals	15/15	15/15	23/25	15/65	3/60	0/10
4 Doses at 24 hr. Intervals	15/15	15/15	32/35	3/65	1/60	0/10
8 Doses at 12 hr. Intervals	15/15	15/15	25/25	26/45	1/40	0/10
12 Doses at 8 hr. Intervals	14/14	15/15	25/25	37/45	1/40	0/25
24 Doses at 4 hr. Intervals	15/15	15/15	25/25	42/45	10/40	1/25

Untreated Controls	1/100
HCl-Injected Mice (24 doses at 4 hour intervals)	23/23
Infected Mice Treated with HCl (24 doses at 4 hour intervals)	0/20

two clear-cut trends are manifested. Ninety-three per cent of the mice are saved on the 4-hourly schedule, 82 per cent on the 8-hourly schedule, and 58 per cent on the 12-hourly schedule. A low of 4 per cent is reached on the 24-hourly schedule, and then successive increases to 23 percent and 50 per cent are seen on the 48-hourly and "96-hourly" schedules respectively.

The toxicity observed was of two main types. The first was an apparent irri-

tation and transient dragging of the injected limb immediately after injection. This occurred in the majority of animals at one time or another during the course of treatment, and was also seen in those mice which had received only 0.1 N HCl. The second type of toxic manifestation was characterized by a delay in onset, and consisted of the development of areas of slough at the injected sites, plus what appeared to be ischemia of the distal portions of the limbs, with atrophy or loss of these distal portions. This second type of reaction occurred in roughly one-third of the animals, and was not seen in any mouse that received only HCl. The involved areas healed without event, and did not seem to play a role in the death of the mice.

It should be noted that the injection of 0.1 N HCl in amounts equivalent to the largest total volume administered, i.e., 24 "doses" at 4-hour intervals, neither killed uninfected mice nor saved infected mice.

B. CONCENTRATIONS OF "TERRAMYCIN" IN PLASMA

Methods. The absence of a chemical method for determining terramycin blood concentrations necessitated the use of microbiological assay to determine the length of time that antibacterial activity persisted in mouse plasma following various single doses of terramycin. It should be understood that in the ensuing discussion "terramycin concentration in the plasma" is used to signify a degree of antibacterial activity, conferred upon the plasma by parenteral administration of the drug, and measured by comparison with similar activity manifested by given amounts of crystalline terramycin in a standard *in vitro* system. This measured antibacterial potency was considered a rough guide to the amount of terramycin present in the plasma of the studied animals.

The procedure involved first the calculation of the total 4-day dose required to save 50 per cent of a group of infected animals (Median Survival Dose, or $S.D._{50}$). This was done by applying the method of Litchfield and Wilcoxon (6) to the data obtained in the therapeutic experiments, and gave the following values:

Regimen	*S D.₅₀ in mg/kg (with 19/20 confidence limits)*
96-hourly	50.0 (42.4–59.2)
48-hourly	61.2 (52.0–72.0)
24-hourly	76.0 (65.6–88.0)
12-hourly	45.6 (40.8–51.2)
8-hourly	40.0 (36.0–44.4)
4-hourly	28.8 (24.0–34.4)

The $S.D._{50}$ for each of the 48-, 24-, and 12-hourly regimens was then divided by the total number of doses that would be given over the 4-day period for that regimen (i.e., 2, 4, and 8 respectively), thus obtaining the individual treatment fraction for each regimen:

Regimen	*Individual treatment fraction (mg/kg)*
48-hourly	30.6
24-hourly	19.0
12-hourly	5.7

Each of these treatment fractions was then injected into the hind limbs of a group of mice, and samples of heart blood drawn into citrate-moistened syringes at various times after injection. For each time interval studied, 0.25 ml. samples of blood from each of 12–15 mice were pooled in a tube containing dried citrate, the blood immediately centrifuged, and the plasma pipetted off and kept frozen at −16°C. until such time as assay was to be performed. Each figure in table 3 therefore represents the mean "blood concentration" of 12 to 15 mice. For the q.24 and q.12 hour schedules, duplicate experiments were run, utilizing separate batches of mice.

The assay method was a modification of that of Tompsett, Shultz, and McDermott (7). Broth, rather than 25 per cent serum, was utilized as diluent, since our experiments indicated that mouse plasma in dilution as low as 1 to

TABLE III

Concentration of "Terramycin" in the Plasma of Mice Following Injection of Individual Treatment Fractions of the S.D.$_{\text{LD}_0}$ Obtained for Various Dosage Regimens

HOURS AFTER TREATMENT	MICROGRAMS OF "TERRAMYCIN"/ML. PLASMA				
	48 Hourly Schedule	24 Hourly Schedule		12 Hourly Schedule	
1	4.0	—	2.0*	1.0	1.2*
2	—	1.5	1.3*	0.7	0.6*
3	2.0	1.2	1.0*	0.4	0.4*
4	1.6	0.6	0.25*	0.4	0.2*
7	0.6	0.3	—	—	—
8	—	—	—	—	<0.2*
12	—	—	<0.2*		
24	<0.4				

— Indicates not done.

* Indicates duplicate runs with different batches of mice.

4 had no appreciable effect on the endpoint. A 10^{-4} dilution of an overnight culture of *Staphylococcus aureus*[6] was used as the test organism. Under these conditions, 0.03 to 0.08 mcg. of terramycin per ml. was found sufficient to "inhibit growth", i.e., prevent turbidity, in standard tubes 8 hours after inoculation. Such standards were run with each plasma assay.

Results. The data are presented in table 3. The degree of reproducibility is evident in the results achieved in the duplicate experiments. It would appear that in the case of the 12-hourly schedule there is at least a 4-hour period between doses when no "terramycin" can be detected in the plasma; in the 24-hourly schedule, at least 12 hours are "terramycin-free"; and in the case of the 48-hourly schedule there is a period of at least 24 hours between doses during

[6] For this strain of *Staphylococcus aureus* the authors are indebted to Dr. Caroline Chandler of the Department of Preventive Medicine, The Johns Hopkins University School of Medicine.

which detectable "terramycin" concentrations cannot be demonstrated in the plasma.

DISCUSSION

The results with this experimental pneumococcal infection of mice indicate that with a constant total dosage and treatment period it makes no difference, for the most part, how frequently the terramycin is administered—the end result in terms of mortality is the same. It is only within a very narrow range that schedule plays a part in determining whether the infected animal will live or die. At the 50 mg. per kg. level, where the relationship is most nicely demonstrated, the minimal effectiveness is achieved with once-daily administration of the drug, with an increase in efficiency when one either administers larger individual doses q.48 hours or "q.96 hours" or smaller individual doses q.4, 8, or 12 hours. In view of the results obtained in Part B. above, one cannot hope to explain the difference in efficacy by relative ability of the various regimens to maintain a continuous blood concentration of terramycin. Differences in total treatment time or total dose cannot be invoked as explanations, since the mice on the 24-hourly schedule do not die until after the 4-day treatment period has been completed. It would therefore seem that any advantage that is obtained from the individually larger dose on the 24-hourly schedule (which is 6 times the size of the individual dose on the 4-hourly schedule) is more than offset by the long lapse of time between injections. The net result of this schedule is inhibition of the growth of the pneumococci sufficient to allow survival of the infected animal while on therapy, but not sufficient to permit eradication of the infection.

The next question to be answered is: Why then the *increased* efficiency with the q.48 and "q.96" hour schedules? If one plots the data at the 50 mg. per kg. level as in figure 1, i.e., survival rate against interval between doses, two lines of regression may be drawn. The first (I) passes through the 4-, 8-, 12-, and 24-hourly points, and has a slope of -4.6, with a standard error of the slope of 0.29. The second line (II) passes through the 24-, 48-, and 96-hourly points, and has a slope of $+0.63$, with a standard error of the slope of 0.048. The standard errors indicate that one may indeed consider these two trends as real. A possible explanation of the relationship of survival to interval between doses is apparent if it is realized that one is also plotting survival rate against size of individual dose, since the latter and the time interval between doses are mutually determinant. An increase in time between injections is easy to visualize as contributing to *decreased* efficiency of treatment, since the organisms are given more time to recuperate from any deleterious effects of the drug. An increase in size of individual dose is easy to visualize as contributing to *increased* efficiency of treatment. A reasonable hypothesis would seem,

therefore, that one is actually viewing the relationship of survival to *interval*, up to q.24 hours, and survival to *size of individual dose* beyond this point.

The fact that the single dose schedule is significantly better than the once-daily-for-four-days schedule is of some interest. Although the time of treatment is not strictly comparable, one would expect any advantage from the difference to lie on the side of the 24-hourly schedule. The results must mean either more efficient bacteriostasis from the one big dose, so that the drug plus the body defenses can rapidly eliminate all the infecting organisms, or an actual bactericidal effect of the drug. In this regard it is of interest that one

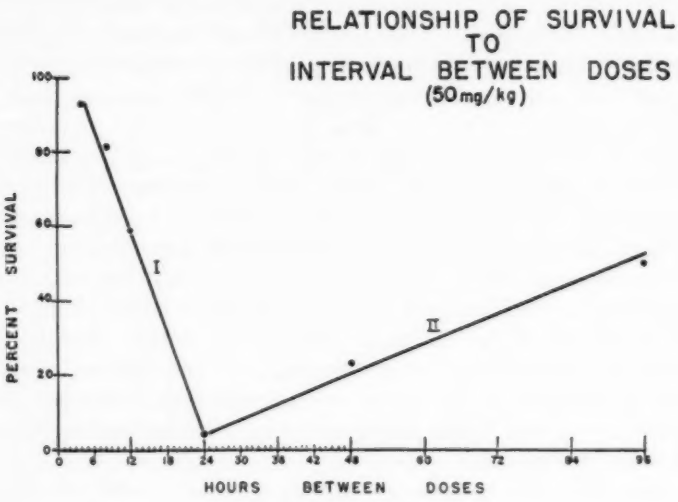


FIG. 1

can demonstrate, *in vitro*, both bacteriostatic and bacteriocidal activity with terramycin, depending on the concentrations employed (3).

The results with the 50 mg. per kg. dose split into two 48-hourly doses deserve some comment. In view of the demonstration that a single dose of terramycin can cure (single dose schedule at 50 and 100 mg. per kg.), and since there is so much "terramycin-free" time between injections given 48 hours apart, the possibility must be entertained that the first dose is curing the animals, and the second dose is contributing nothing to the survival rate. A point in favor of this view is the exceedingly high percentage of deaths occurring on this regimen before the second injection could be administered—94 per cent of the total—or the even higher percentage of deaths (98 per cent) occurring in the first 53 hours after infection, i.e., those mice which either did not receive the second dose or died within a few hours after receiving it. These

data might suggest that most of the animals which lived to get a second dose had already been cleared of all infecting organisms and would have lived without it.

If one now examines the data for the single dose regimen at the 25 mg. per kg. level, which represent the cure rate for single dose treatment with the amount of drug equal to that given in each of the q.48 hour doses at the 50 mg. per kg. level, one would expect the survival rates to be identical for these two regimens, if the above speculation be correct. They are *not* identical, however, being 1.5 per cent and 24 per cent respectively, which are significantly different ($p = <0.01$). Therefore, the second injection in the 48-hourly regimen *is* actually contributing to the cure rate. These data would seem to justify the inclusion of the 48-hourly "blood concentration" data as evidence for the possibility of cure without the continuous maintenance of terramycin concentrations in the blood stream.

It is of interest to compare the results obtained with terramycin with those reported in the literature for other chemotherapeutic agents. It is generally accepted that a continuous blood concentration is required for successful treatment of experimental infections with the sulfonamides (8). The bulk of the data on penicillin and streptomycin indicate that this situation does not obtain for these latter drugs (8). The terramycin data reported here constitute the first attempt to determine whether a continuous blood concentration is required for successful therapy with any of the newer antibiotics, and appear to indicate that terramycin resembles penicillin and streptomycin, rather than the sulfonamides, in this respect.

Much has been made in the past of the relationship between blood concentration of a drug and the amount of drug required to inhibit the growth of the infecting organisms *in vitro*. The recent work of Jackson and Finland (9) is important in this regard. Their demonstration of the tremendous variation possible in "inhibitory concentration", depending on the choice of method of assay, size of inoculum, time of reading, and criteria for end point, plus the well known hazards involved in attempting to transfer *in vitro* data to the *in vivo* situation would seem to render attempts to correlate "inhibitory concentrations" *in vitro* with blood concentrations unrewarding. Utilizing the assay procedure described previously in this paper, but with a 10^{-4} dilution of an overnight culture of the pneumococcus employed in the mouse infection, and a 24-hour reading, 0.2 mcg. of terramycin per ml. was required to inhibit growth. What this may mean in terms of blood concentration of terramycin required to inhibit or kill the organism *in vivo*, especially in view of the demonstration that persistent blood concentrations of drug are not required for cure, would seem at best a matter for idle speculation.

The search for implications of a study of this type for clinical practice is always tempting. Despite the danger in attempting to transfer concepts and

results in experimental infections in animals to human infections, the data may serve as a basis for rational predictions which could be verified or invalidated by clinical trial. First, it would appear that one can cure infections without maintaining continuous concentrations of terramycin in the blood stream. Secondly, a good working hypothesis would seem to be that in the usual clinical use of terramycin, where the susceptibility of the organism was such that the usual daily doses of terramycin would be far in excess of the amount needed to cure the infection, it would make no difference whatever whether the daily dose was given in one good-sized dose or 3, 4, or 6 smaller doses. The limiting factor would probably be toxicity, gastrointestinal or otherwise.

Studies on the metabolism of terramycin in the human (10, 11) indicate that the drug is excreted considerably more slowly by the human than by the mouse. Therefore, even if one were to attempt to reproduce in man the type of regimen seen with 4-hourly doses in mice, the total daily dose would still probably be broken up into 8- or 12-hourly doses. Our data would therefore suggest that rather infrequent doses could be administered safely and that such practice would be based on sound pharmacological principles. Indeed, apparently successful clinical use of terramycin in pneumonia of pneumococcal and mixed bacterial etiology has been reported with doses given as infrequently as q.8 hours (12) or q.12 hours (13).

SUMMARY

1. The relationship of dosage schedule to therapeutic efficiency of terramycin has been studied in an experimental pneumococcal infection of mice.

2. At total dosage levels of 100 mg. of terramycin or more per kg., essentially all infected animals were saved, regardless of dosage schedule. At 12.5 mg. of terramycin per kg. essentially all infected animals died, irrespective of regimen employed. At intermediate dosage levels, this apparent independence of therapeutic efficacy and spacing of doses was not evident, and the 4-hourly administration of drug was slightly superior to other dosage schedules.

3. It is not necessary to maintain a continuous blood concentration of terramycin for cure of this infection.

The authors were greatly helped in their interpretation of data by the generous aid on statistical matters provided by Dr. Margaret Merrell of the Department of Biostatistics, School of Hygiene and Public Health.

BIBLIOGRAPHY

1. Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W. and Kane, J. H. Terramycin, a new antibiotic. Science, 1950, **111**: 85.
2. Terramycin Bibliography, March 15, 1950 to March 15, 1951. Chas. Pfizer and Co., Brooklyn, New York, 1951.

3. Hobby, G. L., Lenert, T. F., Pikula, D., Kiseluk, M. and Hudders, M. E. The antimicrobial action of terramycin. Ann. N. Y. Acad. Sc., 1950, **53:** 266.
4. Bryer, M. S., Schoenbach, E. B., Bliss, E. A. and Chandler, C. A. Treatment of experimental infections with aureomycin. Ann. N. Y. Acad. Sc., 1948, **51:** 254.
5. Regna, P. P. and Solomons, I. A. The chemical and physical properties of terramycin. Ann. N. Y. Acad. Sc., 1950, **53:** 229.
6. Litchfield, J. T., Jr. and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. and Exp. Therap., 1949, **95:** 99.
7. Tompsett, R., Shultz, S. and McDermott, W. The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, Dihydro F, and K. J. Bact., 1947, **53:** 581.
8. Marshall, E. K., Jr. The dosage schedule of chemotherapeutic drugs. Pharm. Rev., 1952, **4:** 85.
9. Jackson, G. G. and Finland, M. Comparison of methods for determining sensitivity of bacteria to antibiotics in vitro. Arch. Int. Med., 1951, **88:** 446.
10. Welch, H. Absorption, excretion, and distribution of terramycin. Ann. N. Y. Acad. Sc., 1950, **53:** 253.
11. Schoenbach, E. B., Bryer, M. S. and Long, P. H. The pharmacology of terramycin in animals and man with reference to its clinical trial. Ann. N. Y. Acad. Sc., 1950, **53:** 245.
12. Timpanelli, A., Huebner, R. D. and McDermott, W. Terramycin in the treatment of pneumococcal and mixed bacterial pneumonias. Ann. N. Y. Acad. Sc., 1950, **53:** 440.
13. Hubbard, W. N., Jr. and Tillett, W. S. Terramycin in the treatment of pneumococcal pneumonia. Ann. N. Y. Acad. Sc., 1950, **53:** 429.

MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

MONDAY, FEBRUARY 11, 1952

Dr. Grafflin: The first paper this evening is by Dr. T. F. Hilbish.

THE SIGNIFICANCE OF CARDIAC MENSURATION BY ROENTGENOLOGICAL METHODS

A study was undertaken to determine the reliability of the various cardiac measurements by radiological methods. Five hundred persons with no history of cardiac disease received teleoroentgenographic chest films. Measurements were made of the transverse, long, and broad diameters and of the frontal plane area of the heart. The mean values, standard deviations and normal ranges for each of these cardiac dimensions were determined. Correlation coefficients were calculated between the various cardiac dimensions and extrinsic factors such as height and weight of the patient as well as the transverse diameter of the chest.

The results of this study indicate the following:

1. The normal range of the various cardiac dimensions is surprisingly large and overlaps the range of abnormality to a considerable degree.
2. The ranges of normalcy of the various cardiac dimensions are not amenable to significant reduction by any of the statistical or technical methods currently available to us.
3. Unidimensional measurements such as the long or broad diameter of the heart are almost as reliable as the more complex duodimensional measurements such as the frontal plane area.
4. All cardiac measurements assume true significance only when it is possible to compare them with previous or subsequent examinations. Only then can a reliable estimation be made of the progression or regression of cardiac disease.
5. Roentgenological cardiac measurements are of limited value and there is little to be gained by masking this fact. Indeed, cardiac measurements can be of value in the study of heart disease only when their true significance is clearly understood by the cardiologist and radiologist.

Dr. Grafflin: Dr. Morgan.

Dr. Morgan: A year or two ago, Dr. Caroline Thomas, when she began working with medical students in an effort to determine some of the cardiac characteristics of young healthy individuals, wished to make X-ray films of these students during the four year period of the study in order to make analyses of cardiac size. She soon became quite concerned over the wide range of the measurements that she obtained and immediately started perusing the literature in an effort to gain some information concerning what this range should be. Much to her surprise, and to ours, too, factual information concerning this material was not available and, at her request, I asked Dr. Hilbish to undertake the study he has reported tonight.

I think that the data which have been collected in the study point out clearly that cardiac mensuration should be regarded with a good deal more caution than has been given it in the past. In many cardiac clinics, a quick estimation of cardiac size is made on the basis of the transverse diameter of the heart relative to the transverse dimension of the thorax.

If there is a discrepancy in which the transverse cardiac diameter is greater than 50 percent of the transverse thoracic diameter, the immediate reaction is that the heart is enlarged. As a result of Dr. Hilbish's studies it is quite evident that considerable enlargement may occur before the heart becomes as large as this.

To emphasize one or two things, I should like to point out that, when one examines the reports of X-ray films on cardiac mensuration, one should not be surprised that the values are 10, 15 or 18 per cent larger or smaller than the average normal. Under these circumstances, the heart may be quite within the normal range. It should also be pointed out that the range of abnormality frequently can overlap the range of normalcy very greatly; cardiac measurements, therefore, are often of limited value. I think that the greatest value in cardiac mensuration occurs when a series of X-ray films are made over a period of time. These films will show the changes in the heart size of the individual and therefore will give an indication of the progress of the individual's cardiac disease.

Dr. Newman: I think that radiologists suffer from the same difficulty as many of us who try to make measurements of the heart. Most measurements show a wide variation in the normal group and many abnormal subjects overlap the normal spectrum. This is true for measurements of venous pressure, cardiac output, heart size, the electrocardiogram and many other methods. Thus the separation of the normal from the abnormal on a statistical basis is always very poor, and is not much help to the clinician. Instead of comparing an individual to a statistical normal scale, I think it would be better to compare the individual with himself under different conditions. When the abnormal heart is put under stress it behaves usually in a clearly abnormal way even though it may look or act normal statistically. For example, after exercise the heart may fail to empty, and the output fails to rise, although the resting output may be within the limits of statistical normalcy. Similarly the electrocardiogram may appear normal in a patient with coronary insufficiency, but will become clearly abnormal after a little exercise.

The point I am making is that the patient must be his own control to a large extent and the direction of change of measurements correlated with function is more accurately diagnostic of abnormality than comparison of measurements on one patient with a statistical group of patients. This is particularly true of the earlier or milder forms of abnormality where the clinician needs the most help.

Dr. Morgan: I would like to ask Dr. Newman what the change is in the cardiac blood volume, under stress, in abnormal hearts and in normal hearts.

Dr. Newman: I think that is something we would like to know. If you take an athletic person and double or triple his cardiac output, there is not much change in the pulse rate, until his volume of blood storage increases as much as two hundred cubic centimeters. I don't know whether you can measure that. In the abnormal person, exercise causes the residual volume to rise much more.

Dr. Morgan: That is an interesting point to follow; 200 cc, however, is not going to increase any one of the cardiac dimensions very greatly; that is, when one considers that in the wide range of normalcy. I think, though, your point is well taken.

Dr. Grafflin: Dr. Hilbish, would you care to comment on this?

Dr. Hilbish: I don't believe I have more to add. I feel much the same as Dr. Morgan on this subject. Our current findings indicate such an overlap between the ranges of normalcy and abnormality of cardiac measurements that it would be very difficult to evaluate small changes in cardiac volume.

Dr. Grafflin: Is there any further discussion? The second paper is by Dr. Vernon Mountcastle.

THE CENTRAL NERVOUS PROJECTION OF SOME FORMS OF DEEP SENSIBILITY

The experiments described were designed to study the central nervous representation of afferent impulses from muscle. In their course we encountered a form of sensibility arising in the deep connective tissue and periosteum of the limbs, the central nervous representation of which could be readily determined. The results of both these groups of experiments are described.

Afferent volleys in isolated muscle nerves were generated by electrical stimuli of varied intensity. The portion of the afferent fiber spectrum conducting the volley was determined by an analysis of the reflex discharge evoked by it into sectioned ventral roots, and recorded there using conventional oscillographic techniques. This information was correlated with the appearance of evoked potentials in various portions of the central nervous system.

Afferent volleys confined to Group I fibers (A-alpha, which innervate stretch receptors in muscle) evoke monosynaptic reflexes at the spinal cord level, and are conducted over central pathways to the anterior lobe of the cerebellum. Such volleys do not evoke responses in the cerebral cortex, nor does direct stretch of muscle. When the stimulus is intense enough to activate the large numbers of smaller Group III fibers in muscle nerves (A-delta, peripheral origin unknown), polysynaptic reflex discharges appear in the ventral root record. Simultaneously, evoked responses of long latency appear in Somatic Areas I and II of the contralateral cerebral cortex, and in Area II of the ipsilateral cerebral cortex, areas known to be devoted also to the representation of cutaneous sensibility. The modality of sensation mediated by these small muscle afferents is uncertain. It may be nociceptive in character.

Light mechanical stimulation of sensory receptors located in deep connective tissue, periosteum, and about joints evokes responses in Somatic Areas I and II of the contralateral cerebral cortex, and Area II of the ipsilateral cerebral cortex. The patterns of representation are more or less coextensive with those for cutaneous sensibility. Conditioning of the somatic afferent system by a volley confined to cutaneous afferents renders the cerebral cortex refractory to a subsequent volley in periosteal afferents for more than 50 msec., and vice versa: there is occlusion between the two.

This form of deep sensibility is relayed to the cerebral cortex via the ventrobasal complex of the thalamus, in somatotopic conformity with cutaneous sensibility.

Preliminary studies using microelectrode recording techniques indicate that within any particular portion of the thalamic relay nucleus, e.g. the thumb area, adjacent cells may each be solely devoted to the transmission of impulses originating in a single type of peripheral receptor, deep or superficial. No interaction between these types has yet been observed at the thalmic level.

Dr. Grafflin: Dr. Bard, would you care to discuss this paper?

Dr. Bard: Dr. Grafflin, I don't think I have very much to add except to point out that this application of the method of recording unitary activity in the central nervous system

is a logical result of the work done 20 or 25 years ago by Adrian and Bronk in their analysis of the relationship of receptor stimulus to afferent nerve discharge. This is a very exciting and provocative stage in the development of our knowledge in afferent systems. When you can get into the thalamus an electrode with a tip only 2 or 3 micra in diameter you are in a position to do something. I went away last summer well realizing that Dr. Rose had long been an accomplished neuroanatomist and a very good neurophysiologist, but when I got back I found he had established himself as a metallurgist. These microelectrodes bear testimony to that fact.

Dr. Grafflin: Dr. Otenasek.

Dr. Otenasek: I sometimes wonder whether it might not be wise to impose some form of academic censorship on the neurophysiologists. For many years now we have seen beautifully executed and controlled studies coming from the northeasternmost corner of the campus. The physiological contributions inherent in these have been widely acclaimed, but unfortunately the clinicians have frequently overstepped the bounds of good judgment in making premature attempts to apply the principles described.

The main difficulty is that there are too few neurosurgeons today who are able to apply the principles of the true scientific method as they are applied in the experiments that have come from Doctor Bard's laboratory. The most striking clinical deficiency is seen in some of the attempts at the surgical relief of pain. It is true that the physiologists have shown that there are primary and secondary pain receptor areas in the cerebral cortices, but no physiologists have presumed to say that they stopped pain, even in their animals, by removal of such areas. Unfortunately the clinicians have presumed to remove these areas in many patients without significant relief of symptoms. It is the clearcut job of the clinician to avoid too excessive and premature application of physiological observations made in laboratory animals.

Dr. Bard: I might say that Dr. Otenasek is entirely correct. His patients, unlike ours can report what they feel. Therefore, I think that it is the neurosurgeons who can best determine what we should do next.

Dr. Grafflin: We will pass on to the third paper, which will be presented by Dr. Jonas Friedenwald.

THE INFLUENCE OF IONIZING RADIATION ON MITOTIC ACTIVITY IN THE RAT'S CORNEAL EPITHELIUM

A study of mitotic activity in the rat's corneal epithelium following X-radiation shows that an inhibition of mitosis occurs after exposure. For the same dose in roentgens the effect is independent of X-ray voltage or of the dosage rate. Over a large dosage range the 50% recovery time is proportional to the dose. This suggests that some substance continuously manufactured during interphase and required in a particular amount for entrance of the cell into mitosis is the radiosensitive substance destroyed by the radiation. Exploration of very low dose effects reveals that a threshold region can be reached at which only a fraction of the cells are affected. The hypothesis that in this threshold range the damage per cell could be accounted for in terms of integral units of damage was confirmed by showing that the data conform to Poisson theory distribution. The number of units of the radiosensitive substance required for the cell to be able to enter mitosis was estimated as approximately one hundred. It may be noted that this is approximately twice the number of chromosomes in the rat's somatic cells.

Dr. Grafflin: This paper is open for discussion.

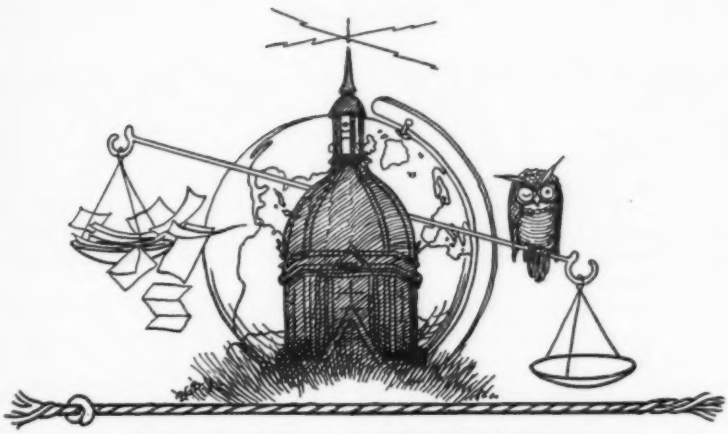
Dr. Rich: I would like to ask Dr. Friedenwald, to explain why he thinks that urethane could not by itself be responsible for some of these effects on mitotic activity.

Dr. Friedenwald: Urethane by itself in a single dose such as we gave does not have any measurable effect on mitotic activity. Urethane plus atropine does. The average interphase time under urethane plus atropine is about twice the normal value and part of the increased sensitivity to X-radiation under those circumstances is attributable to the fact that the whole mitotic activity is running in low gear. This is an advantage from the technical point of our studies. However, we have done studies under ether anesthesia and under no anesthesia, and in both those cases we reached an estimate of the 50% recovery time of a minimal effect as of the order of 1 percent of the total interphase, the same as under urethane and atropine. The phenomena under those circumstances were somewhat more complicated. There is a bounce in mitotic activity following recovery, which makes mathematical analysis more difficult, but the particular feature I am talking about tonight is exhibited under all three circumstances.

Dr. Grafflin: Is there any further discussion?

Before adjourning this meeting I should like to express our warm appreciation to Dr. Hilbish, Dr. Mountcastle, and Dr. Friedenwald for these three excellent presentations.

The meeting is adjourned.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Topics in Physical Chemistry, 2nd edition. By W. MANSFIELD CLARK. 777 pp., $10.00. *The Williams & Wilkins Co., Baltimore, Md.*

It is probably unrealistic to expect medical students to handle all aspects of the subjects covered in *Topics in Physical Chemistry*, but there is no excuse for their not understanding a fair share of the basic principles involved. These principles permit a better understanding of biochemical and physiological reactions and those who expect to deal with these reactions will be the better for this depth of comprehension. Perhaps an example of the use of physical chemical principles in dealing with medical problems will serve a purpose. Soon after the introduction of sulfonamides it was noticed that high anti-bacterial levels of some of these drugs were not attainable without some risk of depositing crystals of the agent in the kidneys. As a solution to this problem Lehr (Proc. Soc. Exptl. Biol. & Med., **64**, 393, 1947) applied the physical chemical principle of additivity in solubility. This principle is simply that the solubility of a non-reacting component is independent of the presence of other components, i.e., a solution saturated with one component can still dissolve other substances. With this in mind Lehr showed that he could obtain high levels of anti-bacterial activity without any danger of intratubular crystal formation by using a mixture of several different sulfonamides each at a level below its saturating concentration. Perhaps this is an unusual case but it illustrates the point.

In Professor Clark's revised edition of his *Topics in Physical Chemistry* the medical student and the graduate scientists in general will find clearly presented many facts of this extraordinary subject. As in the original edition the various subjects are well covered with illustrations, tables of original data, and problems. Although many chapters and subjects have been revised, the extent is not so great as the increased thickness of the book might lead one to expect. Neither is the number of pages a fair measure, for although there are some sixty more pages in the second edition, the author has dropped material from the old text.

Dr. Clark has introduced a brief discussion of the "activity" concept and the Debye-Hückel theory into Chapter 14 on "Electrolytes" while retaining it in Chapter 16 where "Equilibria of Proton Exchanges" is discussed.

In Chapter 21 on the subject of "Oxidation and Reduction," where the author has no equal, he writes with unusual clarity born of first hand experience. This chapter has been considerably expanded over that in the previous edition. A large table of Electrode Potentials of reversible systems occurring in biological fluids will be of great interest and use to many. The author's clear discussion of certain aspects of high energy phosphorylation reactions and clarification of the misused expression "bond energy" will also receive general applause. It may be of some interest to students to know that Professor Clark was probably the first to recognize the important fact that electron transfer is the feature common to most biological oxidation and reductions.

Chapter 30 has an expanded section on chromatography including a brief description of that useful tool, paper chromatography. Although interpretation of results from these procedures was first to follow the simple physical chemical concept of partition, subsequent studies (Moore, S. and Stein, W. H., Annals of New York Acad. of Sciences, **49**, p. 265, 1948) have required qualification of this thought.

To those who have seen the first edition I need hardly add that this book reflects its author's character to a marked degree. It is a lucid but uncompromising presentation of a difficult subject. Professor Clark's choice of examples to illustrate particular principles shows the care of a teacher long familiar with both students and subject. The scholarly development combined with a calculated use of pertinent and often colorful remarks places this book above the usual treatises on physical chemistry.

ROGER M. HERRIOTT

Man on his Nature, 2nd edition (The Gifford Lectures). By SIR CHARLES SHERRINGTON. 300 pp., $6.00. *Cambridge University Press, New York.*

The ever increasing complexity and scope of modern science have led, rather inevitably, to the supremacy of the specialist. No longer able to encompass broad areas of interest in a manner compatible with mastery of those areas, investigators have yielded to the tyrannical demands of science and narrowed their intellectual sphere. Yet one may still find a Whitehead, a Russell, a Williams achieving eminence in non-scientific fields of effort, and maintaining, at least in part, the tradition of the Renaissance man. To such company one must add Sir Charles Sherrington. He is at once a brilliant physiologist, a poet, and a philosopher, and this volume fuses the three images in remarkable and rewarding fashion. Originally a series of lectures delivered by Sherrington (at the age of 76!) in Edinburgh in 1937–38, the material has been revised for this new edition.

The first two chapters are predominantly concerned with the sixteenth century French physician-philosopher Jean Fernel. His times and philosophy are presented in some detail, and serve as an effective contrast to the concepts that follow, although there is as much similarity in the ideas of Fernel and Sherrington as there is contrast.

The remainder of the book deals with Sherrington's closely reasoned approach to the mysteries of life, man, nature, and mind. It is natural that the philosophy of a man whose life has been so much concerned with the scientific method should be built on a materialistic base. But this is no crass, cold materialism. If we have forgotten Lucretius, Sir Charles reminds us that this approach can be poetic, and that it can have concern for the individual and for the good life. The author's intimate knowledge of the workings of the cell, the embryo, the nervous system serve to fill him with wonder and admiration. His description of the nervous control of respiration and the waking of the brain and body (pp. 177–178) is breathtaking poetry of the highest order.

Sherrington's philosophy is also devoid of any paralyzing fatalism. Disturbed by the cruelty of life, by that which "spoils the singing of the nightingale," he firmly believes that man not only must, but *can* work with his fellow-man. "Altruism as a passion, that would

seem as yet Nature's noblest product: the greatest contribution made by man to life." He rejects the elimination of values—"Sunrise and sunset *are* beautiful if one child feels them so. . . . .Shall he not appraise the nightingale and evening star by 'values'?"

At times, indeed, one is struck most forcibly by the feeling that Sherrington is intrigued by speculations that cannot easily be fitted into even that variety of materialistic philosophy which has been called "critical naturalism." At one point, for example, he imagines Nature saying, "Bethink you too that perhaps in knowing me you do but know the instrument of a Purpose, the tool of a Hand too large for your sight as now to compass. Try then to teach your sight to grow."

This book deserves widespread reading by scientist and lay person alike. It is not without faults—the writing is occasionally labored, requiring considerable work on the reader's part in following the author's train of thought—but this seems small enough price to pay for the stimulation and pleasure to be derived from a study of this credo. In these times it is reassuring to hear a declaration of faith in the belief that "the position for reading from Nature's lips what she may have to say of Godhead never yet in the past was what it is for us today."

L. L.

Metabolic Interrelations. Transactions of the Third Conference, January 8–9, 1951. Edited by E. C. REIFENSTEIN, JR. 294 pp., $4.00. *Josiah Macy, Jr. Foundation, New York.*

We are pleased to have been asked to review this volume, since it provides us an opportunity not only to appraise its contents but also to call attention to the unparalleled uniqueness of the current policy of the Directors of the Josiah Macy, Jr. Foundation and its Medical Director, Dr. Frank Fremont-Smith. The latter sums up the present aim of the Foundation: "the promotion of meaningful communication between scientific disciplines." To this end over a dozen annual conferences are held dealing with various phases of medicine of which these Transactions represent one. This volume contains the verbatim give and take between a group of 25 investigators in specialized, though related, fields: anatomy, biochemistry, crystallography, dental anatomy, endocrinology, experimental medicine, experimental histology and physiology, orthopedic surgery, pathology, physiology and radiation biology. It must be evident that a two-day informal presentation of data and discussion is bound to produce provocative reading material.

The title, "Metabolic Interrelations," is somewhat misleading since this volume deals only with problems pertaining to cartilage and bone. As to the content, there are sections on osteogenesis, particularly with respect to the problem of induction; bone destruction; calcification of cartilage matrix; composition of bone salt; effects of certain hormones on bone growth in the rat; deposition of radioactive materials and electron micrography of bone. The presentations of Drs. S. B. Hendricks and H. C. Hodge should be specifically cited, that is, the chemical composition of bone salt (tricalcium phosphate hydrate) which provides a tremendous area for the adsorption of other atoms and molecules (Ca, H, PO_4, CO_3, Na, citrate, etc.).

Dr. E. C. Reifenstein has made an excellent presentation of the materials as Editor of these Transactions.

As one of the participants in this conference, this reviewer may be comewhat biased in regard to the value and stimulation such meetings afford. Dr. Fremont-Smith states in this volume that these conferences are "an experiment." After participating in four such "experiments," this reviewer is quite certain that a principle has been established and hopes that the Josiah Macy, Jr. Foundation is well aware of the contribution it is making to medical investigation and progress.

R. H. F., JR.

Nerve Impulse. Transactions of the Second Conference, March 1–2, 1951, New York. Edited by DAVID NACHMANSOHN. 204 pp., $3.50. *Josiah Macy, Jr. Foundation, New York.*

This is a stenographic report of the Second Macy Conference on Nerve Impulse. These conferences encompass much more than the title implies, for they seem concerned with all phases of neurobiology. The topics and principal discussors in the present volume are as follows: Ionic Problems, Henry B. Steinbach; Excitability, George H. Bishop; Central Excitation and Inhibition, Chandler McC. Brooks and Michelangelo G. F. Fuortes; Anesthetizing Action, Frank Brink, Jr.; Metabolism, J. Folch-Pi.

Other participants are Nachmansohn, Bodian, Curtis, Grundfest, Hoagland, Jasper, Prosser, Quastel, Otto H. Schmitt, Wald, Himwich, Magoun, Shedlovsky, and Irwin B. Wilson.

Like the previous year's volume, this is again a book for experts only. On those topics to which the reader brings a considerable background of knowledge, and on which he is interested in contemporary speculation, this book makes interesting reading. On less familiar topics the presentation is likely to be found disappointingly incomplete, disorganized, and confusing. The confusion is promoted by references to illustrative material shown at the conference but not reproduced in the book, by answers to questions which have apparently been deleted from the record, and by other confusing discontinuities where something, perhaps only a shake of the head, is missing. These may be unavoidable limitations in preparing a book by means of stenographic notes.

It is hoped that in future volumes of this series something will be done to reduce errors of the sort illustrated by the following: 2 figures are transposed in Table III, some references are incorrect, Dr. Bishop's "electrotonic" has been changed to "electronic", and even the institutional association of two of the members of the conference are incorrectly given! These deficiences might well be remedied if proof for each section could be read by the expert responsible for the particular topic, instead of the present procedure of leaving all corrections of proof to the editorial staff.

M. G. L.

A Sex Guide to Happy Marriage. By EDWARD F. GRIFFITH. Introduction by ROBERT LATOU DICKINSON. 352 pp., $3.00. *Emerson Books, Inc., New York.*

This is the first American edition of an English manual which was first published in 1935 and is said to have gone into 25 editions abroad. It is "intended as a guide to those who are preparing for marriage, and those already married who desire practical advice and help." It contains a detailed description of the anatomy and physiology of the genital tracts, illustrated with the characteristic drawings of Dr. Robert L. Dickinson, who also supplies this edition with an introduction. In addition, there are several chapters discussing the problems of the single person, the engaged couple, and the concerns of the married couple in such areas as contraception, abortion, sex education of children, etc.

When the author sticks to anatomical and physiological matters, he is obviously on sure ground and he gives instruction in clear, realistic every-day language that is quite satisfying. However, when discussing the emotional aspects of the relations between the sexes he takes on a tone of moral exhortation which is not half so convincing. Dr. Griffith's psychology of sex is directly derived from that of Havelock Ellis, an origin surely not unworthy but certainly incomplete.

There is a final chapter on marriage counseling in the United States by Dr. Robert W. Laidlaw and Frances W. Dow, and several appendices which give, in excellent detail, the places in this country where family counseling, and birth-control and sterility clinics are

available. An adequate bibliography and index complete this carefully prepared and readable American edition.

J. H.

Practical Therapeutics, 2nd edition. By MARTIN EMIL REHFUSS and ALISON HOWE PRICE. 938 pp., $15.00. *The Williams & Wilkins Co., Baltimore, Md.*

This is a publication of over 900 pages written by a large group of contributors and devoted to a variety of aspects of practical therapeutics. The objective is a very inclusive one. The range of topics is exemplified by the following. There is a section on prescription writing that includes interpretations of symbols and several dozen prescriptions of more common usage with their indications. A brief section on dietetics is then presented. A list of recommended contents for the doctor's bag is published. This rather unusual grouping of information composes most of the first section of the work. The second section is titled "Symptomatic Therapy." In alphabetical order such topics as acute abdominal emergencies, acidoses, anuria, coma, cough and vertigo are listed with brief outlines of possible therapeutic procedures for each. The third and longest section is titled "Treatment for Specific Disorders". Included are such topics as vitamin deficiencies, allergies, endocrinological problems, tuberculosis, Buerger's disease and diabetes. Characteristic and diagnostic features of such a variety of diseases are listed as well as recommended therapy. Charts and drawings are employed extensively. The fourth section is titled "Special Treatment" and includes comments on ocular, parenteral and radiation therapy and gynecological procedures.

In simply listing a few of the topics one is rather overwhelmed with the attempted scope of the work. Although there may be a rôle for such a publication in some phase of medical education it is difficult to find it. The book has inadequate information on a large number of its topics. There is a great deal of repetition between the sections. The scope of the book is so great that little critique seems to have gone into its contents.

The book has little of value to warrant its purchase.

P. F. W.

An Atlas of Normal Radiographic Anatomy. By ISADORE MESCHAN, with the assistance of R. M. F. FARRER-MESCHAN. 593 pp., $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

This volume consists of sixteen chapters. The first of these deals with the historical background and production of x-rays and of the production of x-ray images on film. The second chapter describes the development of bone, the gross and microscopic structure of bone and the general appearance of bony structures as seen on x-ray films. The other fourteen chapters describe the radiographic anatomy of the various body regions and systems such as the skull, respiratory system, gastro-intestinal tract etc. The contributions of newer and more specialized procedures such as angiocardiography, aortography, laminography etc., are adequately covered and are an important feature of the book.

The author employs a systematic approach to radiographic anatomy. First the gross structure of the part or region of the body to be examined is described. This is done by the use of many illustrations accompanied by descriptions which are frequently more illuminating than those found in standard anatomical works. A considerable amount of the information is taken from recent literature, particularly that on the anatomy of the lung and of the heart and great vessels. Next the radiographic projections which best demonstrate the structure under consideration are described along with proper positioning of the part. Finally the finished film is considered in detail and compared with the actual gross anatomy of the part under examination. Considerable attention is given to the anatomical changes

associated with normal growth. Also many tables and charts are included which give the normal ranges of size and position of important structures as seen in roentgenograms. Some of these are the Vastine-Kinney graph for determining displacement of the calcified pineal, the Elsberg and Dykes graph of the interpedicular distances of the vertebra and the Ungerleider and Gubner Nomograms for determining cardiac enlargement. The labelling of several of the diagrams and illustrations is apparently in error. Some that might be mentioned are: Pg. 309, Fig. 202; Pg. 373, Fig. 244; Pg. 444, Fig. 290; and Pg. 445, Fig. 292. These are not sufficiently common however to detract from the value of the volume.

This book has a place beside the view box of the practicing radiologist. It offers a large mass of practical concise information which should be quite helpful in everyday film reading activities.

R. N. C.

Biological Antioxidants. Transactions of the Fifth Conference, November 30–December 1, 1950. Edited by COSMO G. MACKENZIE. 229 pp., $3.75. *Josiah Macy, Jr. Foundation, New York.*

By now the purposes of those meetings popularly known as Macy conferences must be familiar to everyone. No panel has been more successful than the one concerned with biological antioxidants which has moved from vitamin E through peroxides, fat metabolism, general consideration of the mechanisms of enzyme action to, in this fifth conference, a discussion of the biological and chemical effects of radiation. Ordinarily in a highly specialized field the uninitiate are frustrated by a strange vocabulary. Happily, most of the regular conferees seem to have been relative strangers to the language of radiation chemistry and their insistent questions evoked the precise elementary explanation, making this volume good reading and a valuable background. There is appended an index to the transactions of the first five conferences on biological antioxidants.

K. L. Z.

Textbook of Refraction. By EDWIN FORBES TAIT. 418 pp., $8.00. *W. B. Saunders Co., Philadelphia, Pa.*

This is an excellent book which covers considerably more territory than is suggested by the name. In addition to covering the subject of refraction about one-half of the book is devoted to ocular motor anomalies.

The approach to refraction used in the book is primarily physiological. The physiology of the four basic stimulus-response reflex mechanisms that control accommodation is considered. An excellent description of tonic accommodation and the accommodation stimulated by blurred images on the retina is given. The description of the accommodation instituted by the convergence impulse is clear and concise. In addition, there is a detailed description of the accommodation stimulated by the proximity of objects which is described as proximal accommodation. Methods are given for measuring these various types of accommodation and their importance in refractive errors is considered. Very little space is devoted to the geometric optics of refraction but the descriptions of ametropia and its determination are good. There is quite an extensive description of numerous refractive techniques not usually used by ophthalmologists such as static non-cycloplegic retinoscopy and dynamic retinoscopy.

The section dealing with ocular motility is particularly interesting. Here again the approach is primarily physiological with an excellent description of the stimulus-response mechanisms important in causing convergence. The four basic stimulus-response reflexes

described are, 1) tonic convergence, 2) fusional convergence, 3) convergence initiated by accommodation through the harmonic reflex and 4) proximal convergence.

The methods of measuring these various reflexes are described and their importance in the analysis of ocular motor anomalies is pointed out. One very important contribution is the idea of compensated and uncompensated heterophoria. According to this idea the degree of heterophoria must be analyzed in relation to the amount of fusional reserve the ocular motor apparatus shows. If the fusional reserve exceeds by a large degree the amount of heterophoria, the heterophoria is considered compensated and non-productive of symptoms. If the fusion reserve does not exceed the heterophoria by a considerable degree, the heterophoria is then uncompensated, produces symptoms and requires treatment.

The style is clear and concise. This is a book which should find a well deserved place in the teaching of refraction and ocular motility.

W. C. O.

BOOKS RECEIVED FOR REVIEW

Attaining Manhood. Revised and Enlarged Edition. By GEORGE W. CORNER. 92 pp., $1.50. *Harper & Brothers, New York.*

Attaining Womanhood. Revised and Enlarged Edition. By GEORGE W. CORNER. 107 pp., $1.50. *Harper & Brothers, New York.*

The Clinical Use of Fluid and Electrolyte. By JOHN H. BLAND. 259 pp., $6.50. *W. B. Saunders Co., Philadelphia, Pa.*

Connective Tissues. Transactions of the Second Conference, May 24–25, 1951. Edited by CHARLES RAGAN. 190 pp., $3.50. *Josiah Macy, Jr. Foundation, New York.*

Der Einfluss des Nervensystems auf Bau und Tätigkeit der Geschlechtsorgane des Menschen. By PHIL. H. STIEVE. 191 pp., Ganzleinen DM 36. *Georg Thieme Verlag, Stuttgart, Germany.*

Diagnostic Bacteriology, 4th edition. By ISABELLE GILBERT SCHAUB and M. KATHLEEN FOLEY. 356 pp., $4.50. *The C. V. Mosby Co., St. Louis, Missouri.*

Die Transfusion von Konserviertem Blut in der Geburtshilfe und Gynäkologie. By H. SCHWALM. 132 pp., Karton DM 14.70. *Georg Thieme Verlag, Stuttgart, Germany.*

Doctors in Blue: The Medical History of the Union Army in the Civil War. By GEORGE WORTHINGTON ADAMS. 253 pp., $4.00. *Henry Schuman, New York.*

The Internship. (American Lecture Series). By ROSCOE L. PULLEN. 35 pp., $1.25. *Charles C. Thomas, Springfield, Illinois.*

Klinik und Therapie der Vergiftungen. By SVEN MOESCHLIN. 430 pp., Ganzleinen DM 45. *Georg Thieme Verlag, Stuttgart, Germany.*

Klinische Elektrokardiographie. By MAX HOLZMANN. 652 pp., Ganzleinen DM 69.60. *Georg Thieme Verlag, Stuttgart, Germany.*

Liver Injury. Transactions of the Tenth Conference, May 21–22, 1951. Edited by F. W. HOFFBAUER. 320 pp., $3.75. *Josiah Macy, Jr. Foundation, New York.*

The Photography of Patients. (American Lecture Series). By H. LOU GIBSON. 118 pp., $5.50. *Charles C. Thomas, Springfield, Illinois.*

Post-Graduate Lectures on Orthopedic Diagnosis and Indications. Vol. III. By ARTHUR STEINDLER. 284 pp., $8.75. *Charles C. Thomas, Springfield, Illinois.*

Prescription for Medical Writing. By EDWIN P. JORDAN and WILLARD C. SHEPARD. 112 pp., $2.50. *W. B. Saunders Co., Philadelphia, Pa.*

The Prevention of Rheumatic Fever. (American Lecture Series.) By LOWELL A. RANTZ. 66 pp., $2.25. *Charles C. Thomas, Springfield, Illinois.*

Problems of Consciousness. Transactions of the Second Conference, March 19–20, 1951. Edited by HAROLD A. ABRAMSON. 178 pp., $3.25. *Josiah Macy, Jr. Foundation, New York.*

Rheumatic Diseases. Based on the Proceedings of the Seventh International. Prepared by The Committee on Publications of the American Rheumatism Association: CHARLES H. SLOCUMB, Chairman. 449 pp., $12.00. *W. B. Saunders Co., Philadelphia, Pa.*

A Textbook of Orthopedics. By M. BECKETT HOWORTH. In Association with: FRITZ J. CRAMER, DONOVAN J. MCCUNE, A. WILBUR DURYEE, WILLIAM LITTLER and WALTER A. THOMPSON. 1110 pp., $16.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Thyroid. By THOMAS HODGE MCGAVACK. 646 pp., $13.50. *The C. V. Mosby Co., St. Louis, Missouri.*

. RANTZ.

20, 1951.
ion, New

pared by
CHARLES
Pa.
FRITZ J.
WALTER

Co., St.